METHOD AND MATERIALS FOR INTROGRESSION OF NOVEL GENETIC VARIATION IN MAIZE

FIELD OF THE INVENTION

This invention relates generally to the fields of molecular genetics, cytogenetics, and plant breeding. More particularly, it relates to a method for identifying allelic fragments of DNA that correspond to genomic introgression segments of Tripsacum origin, and/or recombinant segments of *Tripsacum*-teosinte chimeric genetic origin, which are stably inherited in wide cross hybrids between *Tripsacum* and teosinte (i.e. wild *Zea* sp.) and the progeny from crosses between maize and *Tripsacum*-teosinte recombinants. These unique DNA fragments are distinct because they are not found in the *Zea* parent, maize (*Zea mays* L.), or teosinte species. Maize is the common name used around the world for the staple cereal grain generally referred as corn in the United States. The two terms are used interchangeably throughout this document. These variant DNA fragments formed as a result of intergeneric hybridization between gamagrass (*Tripsacum* sp.) and teosinte (*Zea* sp.) provide unique markers for assisting selection of desirable traits in maize breeding programs, for detection of target DNA sequences in genetic analyses, and for the identification and transfer of new genes in corn improvement that confer resistance to insect pests and diseases, drought stress tolerance, cold tolerance, perennialism, increased grain yield, totipotency, apomixis, improved root systems, root aerenchyma, ability to grow in anaerobic conditions, tolerance of water-logged soils, tolerance of high-aluminum and acidic soils, improved grain quality, enhanced forage quality, ability to attract nitrogen-fixing bacteria to the rhizoshpere, herbicide tolerance, toxic metal tolerance, adaptability to a CO2 enriched atmosphere and other environmental stresses.

RELATED U.S. PATENT APPLICATION DATA

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BACKGROUND OF THE INVENTION

Molecular Genetics. Genetics is the study of genes and heritable traits in biological organisms. In plant breeding, the goal of molecular genetics is to identify genes that confer desired traits to crop plants, and to use molecular markers (DNA signposts that are closely associated with specific genes) to identify individuals that carry the gene or genes of interest in plants (Morris 1998), to determine the DNA sequences and characterize gene expression and function. A genetic marker is a polymorphism or variant allele that reveals the genetic locus or loci in an individual genotype that is associated with the phenotypic expression of a morphological or anatomical characteristic or a biochemical or physiological process. Variants in DNA and proteins are used as markers in molecular genetics. Molecular variants, or mutant genes, are used in genetic analysis to identify a particular gene and its functional role in precise biological activities and processes. Mutation is the process whereby nucleotide sequences and genes change from the reference form, generally designated wild type, to a different form. In genetic analysis mutants are a valuable source of genotypic variation that allows selection of new phenotypes (Griffiths et al. 1993). Mutations occur either in a sequence of nucleotides, a gene (i.e. DNA sequence that codes for a phenotypic trait), or in the chromosomes (i.e. the hereditary packages that contain specific DNA nucleotide sequences supercoiled with proteins into individual units that can be observed cytologically in the nucleus of a cell, also referred to as

linkage groups). In one type of mutation, the nucleotides that comprise the wild type allele of a gene (i.e. the established reference form that marks a particular locus on a chromosome) are altered resulting in a point mutation. In chromosome mutations, segments of chromosomes, whole chromosomes, or entire sets of chromosomes are rearranged via inversions, translocations, fusions or deletions.

The dogma is that mutations are rare, and most newly formed mutations are deleterious. Data on mutation frequencies for seven genes in maize provide a baseline indicating the rarity of mutations in maize (Stadler 1951). Mutation frequency ranged from 0.000492% (i.e. 492 mutants out of a million gametes) in the red color(*R*) gene; 0.000106% (i.e. 106 out of a million) for the inhibitor of *R* (*I*) gene; 0.000011% (i.e. 11 out of a million) for the purple aleurone (*Pr*) gene; 0.0000024% (i.e. 2.4 out of a million) for the starchy (*Su*) gene; 0.0000022% (i.e. 2.2 out of a million) for the yellow color (*Y*) gene; 0.0000012% (i.e. 1.2 out of a million) for the normal kernel (*Sh*) gene, and 0% (i.e. 0 out of a million for the waxy gene (*Wx*).

Because of the rarity of spontaneous mutations, geneticists and plant breeders typically use mutagens such as chemicals and radiation to increase the frequency of mutation rates in order to increase the number of variant forms that might be useful for genetic analysis and selection of new traits. Another method of inducing mutagenesis in maize is transposon tagging whereby a maize line is crossed with a line containing one of the three systems of transposable elements found in maize. When a transposable element inserts into a gene, it causes a mutation. The reported mutation frequencies for transposable element mutator lines varies from 1 in a thousand to 1 in a million (Chomet 1994). To find a mutation in maize using one of these mutagenic lines, a breeder must screen a minimum of 100,000 plants.

Plant Breeding. Conventional plant breeding is the science that utilizes crosses between individuals with different genetic constitutions. The resulting recombination of genes between

different lines, families, species, or genera produces new hybrids from which desirable traits are selected. Plant breeding is achieved by controlling reproduction. Since maize is a sexually reproducing plant, techniques for controlled pollination are frequently employed to obtain new hybrids. Controlling reproduction in maize involves continually repeating two basic procedures: (1) evaluating a series of genotypes, and (2) self-pollinating or crossing among the most superior plants to obtain the next generation of genotypes or progeny. Controlled pollinations in maize utilize two procedures: (1) detasseling, and (2) hand pollination.

Hybrid corn results from crossing specifically selected parental strains called "inbred lines" (Griliches 1957). The inbreds are produced by self-pollinating for a few years to obtain plants that uniformly express desired traits being selected by the breeder. Inbreds themselves, which are less vigorous due to inbreeding depression, are not suitable for the commercial market. However, when two inbred lines from different heterotic groups are crossed, hybrid vigor develops and the resulting hybrids are far superior to the original varieties. This is the process for development and production of commercial hybrid corn seed.

Maize is a monoecious grass that has separate male and female flowers on the same plant. The male or staminate flowers produce pollen in the tassel at the apex of the maize stalk, and the female or pistillate flowers that produce the grain when pollinated are borne laterally in leaf axils tangential to the stalk. Pollination is accomplished by transfer of pollen from the tassel to silks which emerge from the axillary pistillate ears. Since maize is wind-pollinated, controlled pollination in which pollen is collected from the tassel of one plant and transferred by hand to the silks of the same or another plant, is a technique used in maize breeding. The steps involved in making controlled crosses and self-pollinations in maize are standard practice (Neuffer 1982) and are as follows: (1) the ear emerging from the leaf shoot is covered with an ear shoot bag one or two days before the silks emerge to prevent contamination by stray pollen; (2) prior to making a pollination, the ear shoot bag is quickly removed and the silks cut with a knife to form a short brush,

then the bag is immediately placed back over the ear; (3) also prior to making a pollination, the tassel is covered with a tassel bag to collect pollen; (3) on the day crosses are made, the tassel bag with the desired pollen is carried to the plant for crossing, the ear shoot bag is removed and the pollen dusted on the silk brush, the tassel bag is then fastened in place over the pollinated shoot to protect the developing ear.

Genetic resources for crop improvement include the wild relatives of a particular crop. Maize was the staple grain upon which pre-Columbian civilizations in the Americas were founded. Its wild relatives include six species of wild *Zea,* common name teosinte, that are endemic to Mexico and Guatemala west of the Sierra Madre Oriental mountain range, and twelve to sixteen species of *Tripsacum,* common name gamagrass, that range throughout North and South America from Canada to Chile.

The annual teosintes (*Z. mays* ssp. *mexicana*, *Z. m.* ssp. *parviglumis*, *Z. huehuetenangensis*, *Z. luxurians* and a perennial (*Z. diploperennis*) all have the same chromosome number as maize (n=10) and are cross-fertile with maize. The sixth species *Z. perennis* is a tetraploid perennial whose chromosome number is 2n=40. Normal diploid maize is not cross-fertile with the tetraploid perennial teosinte. However, cross-fertility can be achieved by treating maize to double its chromosome number to 2n=40, which can be crossed with *Z. perennis* to produce fertile 2n=40 hybrids (Shaver 1964). When fifty per cent maize segregates selected from the 2n=40 hybrids, 2n=30 triploid plants that were also fertile were recovered. However, all attempts to derive diploid hybrids between maize and *Z. perennis* failed to produce fertile offspring.

Tripsacum is a polyploid, rhizomatous perennial grass more distantly related to maize that has a different chromosome number (x=18). *Tripsacum* species are highly variable in form, vigor, and ecological preference. Adaptations range from seasonally swampy sites, to sandy soils, to tropical habitats and to near-desert conditions. *Tripsacum,* which is not known to form fertile hybrids with maize or with *Zea* naturally, has valuable agronomic characters that could be exploited for the overall improvement of maize but is

hindered by the problem of cross sterility (Kindiger and Beckett 1990).

The progeny of (maize X *Tripsacum*) obtained by artificial methods have ten maize chromosomes and either 18 or 36 *Tripsacum* chromosomes and are male sterile. Female fertility can be partially restored using special techniques that eliminate most of the *Tripsacum* chromosomes (Mangelsdorf 1974). Plants obtained by crossing *Tripsacum* and maize (*Zea mays* L.) employing *Tripsacum* as the pollen donor have unreduced gametes with a complete set of *Zea* chromosomes and a complete set of *Tripsacum* chromosomes. There is one report of a successful reciprocal cross in which *Tripsacum* was pollinated by maize that required a special culture technique to bring the embryos to maturity, but the plants were sterile (Farquharson 1957). Maize-*Tripsacum* hybrids have been crossed with teosinte to created a trigenomic hybrid that has a total of 38 chromosomes; 10 from maize, 18 from *Tripsacum* and 10 from teosinte. The resulting trigenomic plants were all male sterile with a high degree of female infertility (Mangelsdorf 1974; Galinat 1986).

Based on known crossability relationships between *Zea* and *Tripsacum* and the results of prior crosses between them, the success of the crosses between teosinte and *Tripsacum* resulting in viable, fully fertile plants with chromosome numbers of 2n=20 (Eubanks 1995, 1997) could not have been predicted. Reduction in chromosome number in the interspecific crosses was unexpected based on prior art. The fertility of plants resulting from the cross made both ways with *Tripsacum* as pollen donor and pollen recipient was also unexpected based on prior art.

Although the base chromosome numbers of *Tripsacum* and teosinte are different, x=10 in *Zea* and x=18 in *Tripsacum*, the respective total chromosome lengths of *Tripsacum* and diploid perennial teosinte are almost equal. The total length of the 18 *Tripsacum dactyloides* chromosomes is 492.5μ (Chandravadana et al. 1971), and the total length of the 10 *Zea diploperennis* chromosomes is 501.64μ (Pasupuleti and Galinat 1982). It is not easy to obtain a hybrid plant when crossing *Tripsacum* and teosinte. Hundreds of pollinations are required to obtain a viable seed, and approximately half of seedlings

that germinate die soon after germination. However, as evidenced by cross fertility and chromosome number, when precise alignments occur between homologous regions of the chromosomes of *Tripsacum* and teosinte there is a sufficient degree of pairing to occasionally enable the rare and unexpected success of this cross.

The unexpected fertility of *Tripsacum*-teosinte hybrids, and their cross-fertility with maize, are of great value because they provide opportunity for directly crossing the chimeric recombinants with maize. *Tripsacum*-teosinte hybrids provide a genetic bridge for importing new *Tripsacum* genes not found in maize or the wild *Zeas*, as well as novel genetic material formed in the genomic reorganization between the two species that gives rise to viable, fertile plants that can be crossed with maize using traditional plant breeding techniques.

DNA fingerprinting has revealed that new *Tripsacum* alleles not found in maize or the wild *Zeas* and new recombinant DNA fragments not found in either parent are stably inherited in the progeny of succeeding generations and in crosses with maize. The novel DNA fragments and alleles unique to *Tripsacum* are stably inherited in succeeding generations of maize X *Tripsacum*-teosinte. For purposes herein, unique genetic material refers to regions where new DNA fragments are repeatedly and reliably formed whenever crosses between *Tripsacum* and teosinte produce viable, fertile plants.

Feasibility has been demonstrated in plants derived from crossing *Tripsacum*-teosinte recombinants with maize that are resistant to corn rootworm (*Diabrotica* sp.) and corn borer, are drought tolerant, have properties of perennialism, develop aerenchyma tissue in their roots, and can grow in low pH conditions. Investigation and characterization of other improvements to maize including herbicide tolerance, aflatoxin resistance, and enhanced grain quality are underway.

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SUMMARY OF THE INVENTION

In one embodiment of the invention, there is provided a method for screening a plant to determine whether said plant is a cross between *Tripsacum* and teosinte. In the steps of the method, the total genomic DNA is isolated from the plant; then the genomic DNA is digested with one to five restriction enzymes from the group consisting of *Eco*RI, *Eco*RV, *Hind*III, *Bam*HI and *Msp*I; then the restriction digested DNA is probed with one or more DNA markers selected from the group consisting of the maize nuclear DNA probes, maize mitochondrial DNA probes, and *Tripsacum* DNA probes listed in Table 1; then determining if one or more of the novel recombinant chimeric restriction fragments characterized by the respective marker-restriction enzyme association and fragment sizes listed in Table 2 is present, or if one or more of the introgressed *Tripsacum* fragments characterized by the respective marker-restriction enzyme association and fragment sizes listed in Table 3 is present. To produce a *Tripsacum*-teosinte recombinant plant, a *Tripsacum* plant is pollinated by pollen from a teosinte plant by controlled pollination technique, or reciprocally, a teosinte plant is pollinated by pollen from a *Tripsacum* plant. The resulting intergeneric hybrids are fully fertile and cross-fertile with maize. The hybrid plants are characterized by their utility as a genetic bridge to transfer novel genetic materials into maize and their unexpected chromosome number of 2n=20 instead of 2n=28 or 2n=46. This invention relates to hybrid seed, hybrid plants produced by the seed and/or tissue culture, variants, mutants, modifications, and cellular and molecular components of the hybrid plants that contain novel genetic materials

derived from (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*).

In another embodiment of the invention, there is provided a method for screening a plant to determine whether said plant is a cross between maize and a *Tripsacum*-teosinte hybrid plant. In the steps of the method, the total genomic DNA is isolated from the plant; then the genomic DNA is digested with one to five restriction enzymes from the group consisting of *Eco*RI, *Eco*RV, *Hind*III, *Bam*HI and *Msp*I; then the restriction digested DNA is probed with one or more DNA markers selected from the group consisting of the maize nuclear DNA probes, maize mitochondrial DNA probes, and *Tripsacum* DNA probes listed in Table 1; then determining if one or more of the novel recombinant chimeric restriction fragments characterized by the respective marker-restriction enzyme association and fragment sizes listed in Table 2 is present, or if one or more of the introgressed *Tripsacum* fragments characterized by the respective marker-restriction enzyme association and fragment sizes listed in Table 3 is present. To produce a maize X *Tripsacum*-teosinte plant or the reciprocal *Tripsacum*-teosinte X maize plant, the intergeneric hybrid plant (*Tripsacum* X teosinte) or (perennial teosinte X *Tripsacum*) is crossed with maize by controlled pollination. In the cross, the pollen of (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*) is transferred to maize silks, or maize pollen is transferred to the silks of (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*). This invention relates to hybrid seed, hybrid plants produced by the seed and/or tissue culture, variants, mutants, modifications, and cellular and molecular components of the hybrid plants that contain novel genetic materials derived from maize X (*Tripsacum* X teosinte) or maize X (teosinte X *Tripsacum*).

In another embodiment of the invention, there is provided a method for screening a maize plant to determine whether said plant is a backcross between maize and a (maize X *Tripsacum*-teosinte) hybrid plant. In the steps of the method, the total genomic DNA is isolated from the plant; then the genomic DNA is digested with one to five restriction enzymes from the group consisting of *Eco*RI, *Eco*RV, *Hind*III, *Bam*HI and *Msp*I; then the restriction digested DNA is probed with one or more DNA markers selected from the group consisting of

the maize nuclear DNA probes, maize mitochondrial DNA probes, and *Tripsacum* DNA probes listed in Table 1; then determining if one or more of the novel recombinant chimeric restriction fragments characterized by the respective marker-restriction enzyme association and fragment sizes listed in Table 2 is present, or if one or more of the introgressed *Tripsacum* fragments characterized by the respective marker-restriction enzyme association and fragment sizes listed in Table 3 is present. To produce a backcross hybrid maize plant, the hybrid plant obtained from maize X (*Tripsacum* X teosinte) or maize X (teosinte X *Tripsacum*) is backcrossed to maize. In the backcross, the pollen of the trigeneric hybrid plant is transferred to the silks of one of the original parents (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*) or maize. This invention relates to hybrid seed, hybrid plants produced by the seed and/or tissue culture, variants, mutants, modifications, and cellular and molecular components of the hybrid plants that contain novel genetic materials derived from [maize X (*Tripsacum* X teosinte)] maize or maize X [maize X (teosinte X *Tripsacum*)].

In another embodiment of the invention, there is provided plants and plant tissues produced by the method of crossing maize with a *Tripsacum*-teosinte hybrid that contain novel genetic materials and exhibit beneficial agronomic traits. For example, these plants may contain novel genes for such traits as pest and pathogen resistance, drought tolerance, cold tolerance, water-logging tolerance, improved grain quality, improved forage quality, totipotency, perennialism, tolerance to acidic soils, tolerance to high-aluminum soils, herbicide tolerance, tolerance to toxic metals, enhanced adaptability in a carbon dioxide enriched environment, roots with aerenchyma, and ability to attract nitrogen-fixing bacteria to the rhizosphere. These plants can be employed in recurrent selection breeding programs to select for maize inbred and hybrid lines that exhibit such traits.

For the purposes of this application, the following terms are defined to provide a clear and consistent description of the invention.

Aerenchyma. Formation of large intercellular spaces in the root cortex, i.e. the ground tissue region between the vascular tissue and the epidermis.

Allele. One of the different forms of a gene that can exist at a single locus.

Autoradiography. A process in which radioactive materials are incorporated into cellular components, then placed next to a film or photographic emulsion to produce patterns on the film that correspond to the location of the radioactive compounds within the cell.

Electrophoresis. A technique for separating the components of a mixture of molecules (proteins, DNAs, or RNAs) in an electric field within a gel matrix.

Genetic markers. Alleles used as experimental probes to keep track of an individual, a tissue, a cell, a nucleus, a chromosome, or a gene.

Gene. The fundamental physical and functional unit of heredity that carries information from one generation to the next. The plant gene is "a DNA sequence of which a segment is regularly or conditionally transcribed at some time in either or both generations of the plant. The DNA is understood to include not only the exons and introns of the structural gene but the cis 5′ and 3′ regions in which a sequence change can affect gene expression" (Neuffer, Coe and Wessler 1997).

Genotype. The allelic composition of a cell - either of the entire cell or, more commonly, for a certain gene or a set of genes of an individual.

Hybrid plant. An individual plant produced by crossing two parents of different genotypes or germplasm backgrounds.

Inbred. A plant that has been self pollinated or sib mated.

Inversion. A chromosomal aberration in which the order of a chromosomal segment has been reversed.

Linkage group. A group of genes that have their loci on the same chromosome.

Locus. The place on a chromosome where a gene is located.

Molecular genetics. The study of the molecular processes underlying gene structure and function.

Mutagen. An agent that is capable of increasing the mutation rate.

Mutation. (1) The process that produces nucleotide sequences, genes, genetic elements, or chromosomes differing from the wild-type. (2) The nucleotide sequences, genes, genetic elements, or chromosomes that result from such a process.

Phenotype. The observable properties of an organism that are genetically controlled.

Plant breeding. The application of genetic analysis to development of plant lines better suited for human purposes.

Polymorphism. The existence of two or more distinct, segregating forms in a population.

Probe. Defined nucleic acid segment that can be used to identify specific molecules bearing the complementary DNA or RNA sequence, usually through autoradiography.

Restriction enzyme. An endonuclease that will recognize specific target nucleotide sequences in DNA and cut the DNA at these points; a variety of these enzymes are known and used extensively used in genetic engineering and molecular biology.

RFLP. Refers to restriction fragment length polymorphism of a specific size determined by its molecular weight in kilobases that is visualized on a Southern blot when a radiolabelled DNA probe of a specific sequence of known bases hybridizes to the fragment that contains that particular DNA sequence. RFLPs are considered to represent an allele of a gene. When they have been mapped to precise chromosomal loci as in maize, they provide a highly reliable fingerprinting method for precision genotyping of individuals.

Robertsonian fusion. A chromosomal aberration that involved the fusion of long arms of acrocentric chromosomes at the centromere.

SSRs. Simple sequence repeat polymorphisms are intergenic tandem repeats of 2 to 6 base pairs that are amplified by polymerase chain reaction (PCR) using primers complimentary to the flanking regions of the repeats. The PCR products are separated by electrophoresis, and the codominant polymorphisms are visualized as different bands on the gel. SSR variability can be scored as accurately and reliably as RFLP polymorphisms. SSRs are rapidly becoming the molecular markers of choice for genotyping, as well as for identifying and mapping genes and assessing genetic diversity.

Southern blot. Transfer of electrophoretically separated fragments of DNA from the gel to an absorbent surface such as paper or a membrane which is then immersed in a solution containing a labeled probe that will bind to homologous DNA sequences.

Totipotency. The ability of a cell to proceed through all the stages of development and thus produce a normal adult.

Translocation. A chromosomal mutation associated with the transfer of a chromosomal segment from one chromosome to another.

Wild type - refers to a reference and it can mean an organism, set of genes, gene or nucleotide sequence. For purposes herein the wild type refers to the parents of hybrid progeny.

DESCRIPTION OF THE DRAWING

Figure 1 is a schematic drawing of the 10 linkage groups of maize. The open circles represent approximate positions of the centromeres. The relative positions of the RFLP marker probes that were used to DNA fingerprint recombinant plants derived from crossing *Tripsacum* and teosinte, plus plants derived from crossing maize with *Tripsacum*-teosinte recombinants, are indicated on each of the ten maize chromosomes. Molecular markers at loci where stable, heritable variant fragments that are not found in either parent are underscored, and markers that indicate where new alleles from a *Tripsacum* parent that are not found in *Zea* have been inherited in the *Tripsacum*-teosinte recombinant progeny and in crosses between maize and *Tripsacum*-teosinte are italicized.

Figure 2 is a schematic drawing of the 10 linkage groups of maize. The open circles represent approximate positions of the centromeres. Corresponding SSR marker probes are listed beneath the RFLP marker probes used to DNA fingerprint recombinant plants derived from crossing *Tripsacum* and teosinte, plus plants derived from crossing maize with *Tripsacum*-teosinte recombinants.

DETAILED DESCRIPTION OF THE INVENTION

The principles and techniques that were used to detect the chimeric genetic material and novel *Tripsacum* alleles are commonly used to fingerprint crop varieties (Kresovich et al. 1993). First DNA is extracted and isolated from plant samples; then the DNA is cut into fragments using restriction enzymes that cut at precise nucleotide sequences; the fragments are then separated by size, i.e. molecular weight, on an agarose gel by electrophoresis; the DNA is then denatured, i.e. separated into single strands, and transferred to a filter or membrane that binds single-stranded but not double-stranded DNA, a method referred to as Southern blotting. The restriction fragments are immobilized on the filter in the same way they are positioned on the electrophoretic gel. The membrane is then incubated

in a solution containing multiple copies of a radiolabeled probe for a particular DNA sequence that has been mapped to a certain chromosomal locus or loci in the maize genome. The probe hybridizes to homologous DNA sequences to reveal the distinctive bands of specific molecular weight sizes that are formed by a particular restriction enzyme/probe combination in any individual plant. The bands, i.e. the restriction fragment length polymorphisms, are then visualized in the resulting autoradiograph. Like a bar code which the RFLP bands resemble, they precisely identify the genotype of individual plants. This method of RFLP genotyping provides information necessary to distinguish between plants whose genetic composition may differ only slightly. This DNA fingerprinting technique permits the unambiguous identification of genotypes (Melchinger et al. 1991; Messmer et al. 1993). Fingerprinting profiles are routinely used for genetic identity analysis to classify closely related materials, estimate genetic distances, determine paternity, and complement conventional pedigree records in commercial hybrid production (Smith and Smith 1992).

Although maize contains many duplicate genes, it is generally thought of as a diploid organism in which the progeny of maize hybrids inherit one allele for a trait from one parent and another allele for that trait from the other parent. In the DNA fingerprint of a single gene that is not duplicated elsewhere in the genome and the offspring inherit the same polymorphism marked by a molecular probe that maps to the specific region of the particular chromosome to which the trait being investigated has been mapped from both parents, they will be homozygous for that particular trait and a single band will be seen on the autoradiograph. If the progeny inherit different polymorphisms from each parent plant, they will be heterozygous at that locus and two bands will be detected on the autoradiograph, one band from one parent and a different band from the other parent. Multiple bands are seen at more complex loci involving gene duplication. In general, the offspring of two parents can be identified by comparing their DNA fingerprints to those of the parents because progeny exhibit a combination of bands from both parents. Sometimes, however, the progeny of known parentage exhibit

a band or bands that are not found in either parent. Such novel bands may arise from mutations in the nucleotide sequences or from chromosomal mutations that cause genomic reorganization such that some RFLP bands will be different from both of parents (Griffiths et al. 1993).

Such mutant or novel rearrangements in the genetic material are revealed by comparative analysis of the RFLP bands of the parent plants and hybrid progeny. Bands present in the offspring not found in either parent indicate regions of the genome where novel genetic material has arisen, i.e. mutations have occurred. As stated above, mutations are rare, and in most cases deleterious. Broadly speaking among all organisms, mutation rates vary and they range from 1 in 1,000 to 1 in 1,000,000 gametes per generation depending on the gene involved (Curtis and Barnes 1989). For example, each human with approximately 100,000 genes is expected to carry 2 mutant alleles. The unique restriction fragments of the *Tripsacum*-teosinte hybrids occur at 148 out of 176 loci and are unprecedented in their high mutation rate. Furthermore, the novel polymorphisms are stably inherited in succeeding generations of *Tripsacum*-teosinte progeny and of maize X *Tripsacum*-teosinte progeny. In addition to the rarity and usual deleterious effect of mutations, a basic biological tenet is that mutations occur at random or by chance (Lewin 1997). In a study of spontaneous mutation rates to new length alleles at tandemly repeated loci in human DNA (Jeffreys et al. 1988) mutations arose sporadically and there was no clustering of mutations within a family. Siblings never shared a common mutant allele. Therefore, it is unexpected that the same mutations would recur not only among siblings but among hybrids of different parentage. Thus it is remarkable and unexpected that the same unique polymorphisms are repeatedly found in hybrid progeny derived from crossing different *Tripsacum* and different teosinte parent plants (see Table 2), and that those same novel restriction fragments are stably inherited in crosses between *Tripsacum*-teosinte hybrids and maize (see Table 2). These unique RFLPs provide a rich new source of variant genetic material for selection in corn improvement.

In molecular assays performed by Linkage Genetics, Salt Lake City,

Utah, and Biogenetics, Inc., Brookings, South Dakota, DNA was isolated from different F_1, F_2 and F_3 hybrids between *Tripsacum* and teosinte, the parents of these hybrids, W64A and B73 maize inbred lines, as well as F_1, F_2, F_3 and F_4 hybrids between maize and *Tripsacum*-teosinte. The protocol for DNA isolation, restriction enzyme digestion, Southern blotting, probe hybridization, and analysis of autoradiographs has been described by Helentjaris et al. (1986). Internal standards of known molecular weights and a ladder were included in the gels to characterize molecular weights of the bands and facilitate scoring accuracy and analysis.

Total genomic DNA from the individual parent and hybrid plants was digested with from at least one of five different restriction enzymes, *Eco*RI, *Eco*RV, *Hin*dIII, *Bam*I, and *Msp*I, then transferred to Southern blots, and probed with 176 publicly available DNA markers which include a majority of maize nuclear DNA probes mapped to the ten linkage groups of maize (Gardiner et al. 1993), six maize mitochondrial probes, and some *Tripsacum* (tda) probes for which the loci have not yet been mapped to the maize genome. The molecular markers on the genetic linkage map of maize were mapped by recombinational analyses based on proof of the identity of a clone. Thus each locus represents a gene based on clone identification (Neuffer, Coe and Wessler 1997). The 176 molecular markers that were employed in DNA fingerprinting of parent species, *Tripsacum*-teosinte hybrids, and (maize X *Tripsacum*-teosinte) are listed in Table 1. Figure 1 depicts the orders and approximate locations of the mapped probes on the ten maize chromosomes (cf. Neuffer, Coe and Wessler 1997). A large number of the probes reveal bands that are not present in either parent of a particular progeny. These novel bands signal loci where mutations occurred in the process of intergeneric hybridization. Their approximate mapped loci on the ten chromosomes of *Zea* are shown in Figure 1, and they are indicated in Table 1 by underscoring. There are also loci where *Tripsacum* polymorphisms are present in *Tripsacum*-teosinte hybrids that were not present in the genotyped maize lines and other teosinte species. These unique *Tripsacum* polymorphisms can be used to screen for introgression of *Tripsacum* alleles in maize via the *Tripsacum*-teosinte genetic bridge.

They are italicized in Table 1 and Figure 1.

Crosses have been made using seven different *Tripsacums* including three accessions of *Tripsacum dactyloides*, one from Santa Claus, IN (4n=72), one from Hilltop Experiment Station, Bloomington, IN (4n=72), and one from Manhattan, KS (2n=36); *Tripsacum laxum* (CEL 48770)from Veracruz, Mexico, *Tripsacum peruvianum* (DHT-66-13-01) from San Martin, Peru, *Tripsacum manisurioides* 37553 from Woodward, OK, *Tripsacum floridanum* MIA34719 from Florida, and *Tripsacum* sp. from Nabogame, Sonora, Mexico. The *Tripsacums* have been crossed with teosinte plants of *Zea diploperennis* originating from different populations in Jalisco, Mexico; plants 3-7 and 3-3 from a population in Upper las Joyas, Sierra de Manantlan, Iltis, Nee and Guzman accession number 1250, January 1979, and plant 2-4 from a La Ventana population, R. Guzman Accession number 777, December 14, 1977, and with (maize X *Tripsacum*-teosinte) hybrids. The *Tripsacum*-teosinte hybrids included in Tables 2 and 3 are: (1) Sun Dance, *Zea diploperennis* 3-7 X *Tripsacum dactyloides* (2n=72); (2) Tripsacorn, *Tripsacum dactyloides* (2n=72) X *Zea diploperennis* 3-3; (3) Sun Star, *Zea diploperennis* 2-4 X *Tripsacum dactyloides* (2n=36); (4) Sun Devil, *Tripsacum dactyloides* (2n=72) X *Zea diploperennis*; (5) 20A, *Zea diploperennis* 2-4 X *Tripsacum dactyloides* (2n=72). There have been multiple crosses between the maize inbred lines W64A, B73 and A188 with various *Tripsacum*-teosinte hybrids. Hybrids between *Tripsacum*-teosinte and maize included in Tables 2 and 3 are: 64SS (W64A X Sun Star), 64TC (W64A X Tripsacorn), 2019 (B73 X Tripsacorn), 4021 (B73 X Tripsacorn), 3024 (B73 X Tripsacorn), 3028 (B73 X Tripsacorn backcrossed to Tripsacorn), 3125 (W64A X Tripsacorn), 4126 (W64A X Tripsacorn), 3029 (B73 X Tripsacorn), 4029 (B73 X Tripsacorn), 10 individuals of TC64 (Tripsacorn X W64A), 7022 (TC64 backcrossed to Tripsacorn), 7024 (Tripsacorn X W64A), 9094 X 7009 (an advanced maize line in a B73/W64A maize background introgressed with Tripsacorn and Sun Star), 97-5 X 97-1 (an advanced maize line in a B73/W64A maize background introgressed with Tripsacorn and Sun Star), and V70 (an advanced maize line in a W64A/A188 maize background introgressed with Tripsacorn and Sun Star). Other hybrids include 20B, *Zea diploperennis* 2-4 X *Tripsacum dactyloides* (2n=72); Devil Corn, a

cross between Sun Devil and Tripsacorn; [(7022 X Devil Corn) X *Tripsacum laxum*]; 7022 X *Tripsacum manisurioides;* TC64#5 X Nabogame *Tripsacum* sp.; TC64#5 X *Tripsacum floridanum*, and (7022 X Devil Corn) X *Tripsacum peruvianum*.

Tables 2 identifies the molecular marker loci associated with novel restriction fragments, indicates their molecular weight, and specifies in which *Tripsacum*-teosinte hybrids and [maize X (*Tripsacum*-teosinte)] lines they occur. Table 3 identifies the molecular markers associated with unique Tripsacum RFLPs, indicates their molecular weight, and specifies their inheritance in the *Tripsacum*-teosinte hybrids plus exemplary (maize X *Tripsacum*-teosinte) lines in which they are found.

In order to determine which *Tripsacum* polymorhphisms are present in *Tripsacum*-teosinte hybrids that are not present in other *Zeas*, 5 to 13 individuals from populations of two modern maize inbred lines, B73 and W64A, four indigenous Latin American maize races, Nal Tel (Yuc7), Chapalote (Sin), Pollo (Col 35 ICA), and Pira (PI44512), and the six wild *Zeas*, *Z. mexicana* (PI566683 and PI566688), *Z. parviglumis* (PI384061 and PI331785), *Z. luxurians* (PI306615), *Z. huehuetenangensis* (Ames21880), *Z. diploperennis* and *Z. perennis*(Ames 21875), were DNA fingerprinted with the probes in Table 1 and Figure 1. The molecular marker loci are identified by the specific probe/restriction enzyme combination and molecular weight. Table 4 gives the molecular weights of parental RFLPs for comparative reference.

The novel genetic materials, which include the new restriction fragments formed in the wide cross genomic reorganization and unique polymorphisms from *Tripsacum* not found in maize or the wild *Zeas*, have been shown to be stably inherited in three generations of *Tripsacum*-teosinte hybrids, and eight generations of *Tripsacum*-teosinte hybrids that were crossed with maize. The unique *Tripsacum* polymorphisms and recombinant chimeric RFLPs, their heritability in succeeding generations of *Tripsacum*-teosinte hybrids, and their transmissibility to maize is unprecedented and unexpected based on prior art. These novel DNA fragments have utility for genetic analysis of *Zea*, and selection of new variant alleles that may

enhance traits such as insect and disease resistance, drought stress tolerance, cold tolerance, herbicide tolerance, perennialism, increased grain yield, totipotency, apomixis, better root systems, tolerance of water-logged soils, tolerance of high-aluminum and acidic soils, improved grain quality, and improved forage quality. When these novel RFLPs co-segregate with crop improvement traits, they can be successfully employed in recurrent selection breeding programs for early and rapid screening of plants carrying the desired trait. They are also important for identifying the regions of the genome where the genes for the trait reside.

Examples of the application of these molecular markers for genetic analysis and marker-assisted breeding are described in regard to identification of marker loci associated with two traits that are characteristic of *Tripsacum* and have been transferred into maize via the *Tripsacum*-teosinte bridging cross. They include resistance to the insect pest corn rootworm (*Diabrotic virgifera* Le Conte), and formation of aerenchyma in the roots. Aerenchyma tissue consists of large spaces in the root cortex that allow movement of oxygen from the aboveground plant tissue to the roots, an adaptation to anaerobic environments (Comis 1997). Aerenchyma allow the roots to penetrate deep in the soil below the hard pan which greatly enhances drought tolerance. It allows the plant to survive in saturated soils.

Genomic DNA isolated from leaves of *Tripsacum*-teosinte hybrid plants and *Tripsacum*-teosinte X maize hybrid plants that demonstrated resistance to corn rootworm in insect bioassays was subjected to RFLP genotyping as described above. In Table 2 the Tripsacum-teosinte hybrids that exhibit rootworm resistance are Tripsacorn, Sun Star and 20A, and the Tripsacum-teosinte X maize plants that were resistant to corn rootworm are 2019, 3024, 3028, 3125, 4126 and TC64. The fact that the *Tripsacum*-teosinte hybrid called Sun Dance is not resistant provides a unique opportunity to simplify genetic analysis and determine the molecular markers and chromosomal regions to which this trait may be assigned without having to map a large segregating population. This can be done by examining all the unique polymorphisms in Tables 2 and 3 and identifying which ones are found only in Tripsacorn, Sun Star, 20A, 2019, 3024, 3028, 3125, 4126 and

TC64. Since only one molecular marker satisfies this requirement, UMC103 on the short arm of chromosome 8, it is clearly a marker for rootworm resistance. However, since the trait is not expressed in a 3:1 ratio according to simple Mendelian inheritance, and the trait is either expressed in lower frequencies than expected, or expression may be lost in subsequent generations, more than one loci are affecting expression. Although 20A exhibited rootworm resistance in an insect bioassay, it has never been employed in crosses to maize. Therefore, it is assumed the other loci involved in expression of rootworm resistance must be found in Tripsacorn, Sun Star, 2019, 3024, 3028, 3125, 4126 and TC64. There are three additional candidate loci that have a restriction fragment found only in the rootworm resistant hybrids: BNL5.37 which marks a locus on the long arm of chromosome 3, UMC28 on the long arm of chromosome 6, and UMC95 on the long arm of chromosome 9. This information allows the screening of young seedlings for rootworm resistance without having to go through time-consuming, labor intensive insect bioassays. A small amount of leaf tissue can be used to isolate the genomic DNA from individual plants. The sample can be assayed by RFLP genotyping using the respective enzyme/probe combinations for those four loci or it can be done more rapidly by isolating genomic DNA from small amounts of leaf tissue and genotyping by polymerase chain reaction (PCR) with primers for SSR (simple sequence repeat) markers that have been mapped to corresponding positions as the RFLP markers on the maize chromosomes (see Figure 2 and Table 5). Plants with two of these marker loci polymorphisms exhibit a degree of resistance to corn rootworm that is equal to or better than the industry standard root rating of 3 for efficacy of insecticide control. Plants with three or more of these RFLP markers have root ratings of 1 or 2 on the Hills and Peters (also referred to as Iowa) scale and are highly resistant (Eubanks 2002).

Simple sequence repeat polymorphisms (SSRs) are rapidly becoming the molecular markers of choice for genotyping, as well as for identifying and mapping genes (Senior et al. 1998), and assessing genetic diversity (Liu et al. 2000). SSRs are intergenic tandem repeats of 2 to 6 base pairs that are amplified by polymerase chain

reaction (PCR) using primers complimentary to the flanking regions of the repeats. The PCR products are separated by electrophoresis, and the codominant polymorphisms are visualized as different bands on the gel. SSR variability can be scored as accurately and reliably as RFLP polymorphisms (Smith et al. 1997). Advantages for employing SSRs instead of RFLP markers for marker assisted breeding are they are less labor intensive, less time-consuming, more cost effective, permit rapid, high through-put screening, and require much smaller quantities of DNA. To assess feasibility of using SSRs for marker assisted selection of rootworm resistance a pilot study using 35 SSR markers was conducted to see if they would also amplify the DNA of *Tripsacum* and *Tripsacum*-teosinte hybrids. In addition to producing distinct polymorphisms that were inherited from both the *Tripsacum* and the *Zea* parents, novel SSR bands were also observed in the *Tripsacum*-teosinte recombinants and crosses between *Tripsacum*-teosinte hybrids and maize. SSR markers that map to the same genetic loci as the RFLP markers employed to fingerprint the *Tripsacum*-teosinte hybrids are listed in Table 5 and indicated beneath each corresponding RFLP marker in Figure 2. The corresponding SSR markers for the RFLP markers for rootworm resistance are bnlg2235 for UMC103 on the short arm of linkage group 8, dupSSR23 for BNL5.37 on the long arm of linkage group 3, phi123 for UMC28 on the long arm of linkage group 6, and bnlg1714 for UMC95 on the long arm of linkage group 9. The application of SSR marker assisted breeding will greatly facilitate commercial development of maize with natural rootworm resistance imparted from resistant *Tripsacum*-teosinte recombinants.

In addition to rootworm resistance, another application for using these novel RFLP fragments to select special traits is in regard to transferring constitutive aerenchyma to the roots of maize. Aerenchyma refers to large intercellular spaces in plant tissue that permit internal gas transport between the leaves and roots, and serve as a reservoir of oxygen required for respiration under anaerobic conditions (Esau 1977). Aerenchyma is a common feature of wetland and aquatic plants (Justin and Armstrong 1987), and it occurs in some species adapted to drier environments. Another important function of aerenchyma is diffusion of oxygen into the rhizosphere for oxidation

of soil components toxic to plant growth (Armstrong 1979; Drew and Stolzy 1996). Some plants have constitutive aerenchyma that forms early in development. Other plants may gradually develop aerenchyma in response to flooded soil conditions (Justin and Armstrong 1987). The roots of *Tripsacum dactyloides* possess constitutive aerenchyma (Ray et al. 1998). The air-filled passages in the roots enable gamagrass to grow in saturated soils and to penetrate compacted layers so it can tolerate both floods and droughts (Clark et al. 1996; Foy 1996; Ray et al 1998). The roots can grow deep into subsoils to tap water reserves. Since subsoils are highly acidic, aerenchyma appears to be associated with gamagrass' strong aluminum tolerance (Clark et al. 1996; Foy 1997). Examination of the roots of the *Tripsacum dactyloides* and *Zea diploperennis* parents, and Sun Dance Genetics F_1 hybrids under a low power light microscope reveal well developed aerenchyma in all *Tripsacum* parents but none in any of the teosintes. Aerenchyma is present in Tripsacorn roots but not in those of Sun Dance, Sun Star or 20A. The transfer of constitutive aerenchyma into corn will enhance broad environmental stress tolerance in the world's most widely grown crop. Benefits of commercial development of this technology for American producers, as well as growers worldwide, will be reduced vulnerability to weather extremes of drought as well as the opposite problem of excessive rainfall and standing water. Broad environmental benefits will be reduction of aquifer depletion from irrigation and reduced pollution of waterways and groundwater from irrigation runoff.

Upon examination of a series of roots, aerenchyma was observed in other hybrids including Devil Corn, Sun Devil, 7022, 5 plants of 7022 X Devil Corn, B016, 6021, 4 plants of *Zea diploperennis* X *Tripsacum laxum*. Two out of three F_1 plants of Tripsacorn X W64a had aerenchyma. One of two B73 X Tripsacorn plants had intermediate expression of aerenchyma and the other plant had none. This indicates aerenchyma has simple co-dominant inheritance. In a population of 24 SDG058 plants in a breeding program selecting for strong drought tolerance, all had aerenchyma. SDG058 is derived from a B73 X Tripsacorn (ref. 2019 in Tables 2 and 3). Fifteen had well developed aerenchyma indicating they are homozygous for the trait.

Roots of plants from three other (*Tripsacum*-teosinte X maize) hybrid lines that were not selected for drought tolerance (9094 X 7009, 00-2-17, and 99-16-3 did not develop arenchyma. This confirms that the presence of root aerenchyma is contributing to drought tolerance in line SDG058.

SDG058 exhibited strongest drought tolerance when compared to the publicly available corn inbred W64A, the corn parent of SDG058, in controlled environment water deficit experiments. In each of three experiments, 24 plants of each line (12 treatment and 12 control) were planted in 5 gallon pots and placed in the growth chambers in a randomized block design. The plants were watered twice daily until initiation of the water deficit regimen at 42 days after planting, the most critical period in the reproductive and flowering cycle affecting grain yield. The drought period was monitored graivmetrically by weighing the pots daily until they reached a minimum 30% reduction in plant available water. Calculated in pilot tests, this equilibrates to 20% reduction in pot weight. The drought treatment period in these experiments was 5 days with no water and averaged to approximately 30% reduction in pot weight or around 45% reduction in plant available water, a strong drought stress for corn. Grain dry weight was the measure for assaying the degree of drought tolerance. The average SDG058 grain dry weight of plants under drought stress was 198 g per plant. In contrast, the drought stressed W64A corn plants had a yield of 125.2 g per plant. Under drought stress the SDG058 hybrid line outperformed W64A by about 37% greater yield. W64A does not have aerenchyma in its roots. All of the SDG058 plants have root aerenchyma.

Since aerenchyma is present only in Tripsacorn and not in the other *Tripsacum*-teosinte crosses, unique polymorphisms in Tables 2 and 3 found only in Tripsacorn will signal potential markers for this trait. Aerenchyma is present in the maize X *Tripsacum*-teosinte plants designated number 2019, 3028 and TC64 in Tables 2 and 3. Logically, the aerenchyma trait will be associated with any marker for unique polymorphisms found only in Tripsacorn, 2019, 3028 and TC64. The only possible marker locus candidate that has an RFLP fragment found exclusively in the hybrids with constitutive

aerenchyma is BNL8.32 on the long arm of linkage group 7. Therefore it is concluded that the gene for aerenchyma was transferred to the long arm of *Zea* chromosome 7. The corresponding SSR marker for the BNL8.32 locus is bnlg2235.

The present invention provides a method of screening plants to determine if they are crosses between *Tripsacum* and teosinte by isolating their total genomic DNA, digesting the DNA with restriction enzymes, transferring it to Southern blots and probing it with mapped molecular markers to determine the presence of one or more novel or unique RFLPs as defined by probe-enzyme combination and molecular weight. The term "plant" as used in this application refers to the whole plant as well as its component parts, e.g., flowers, roots, fruits, stems, rhizomes, pollen. The crosses are performed using standard plant breeding techniques for controlled pollinations known in the art. Some of the *Tripsacum*-teosinte hybrid plants that are perennials and reproduce asexually as well as by seed have been described in the following plant patents: PP No. 9,640 issued September 3, 1996; PP No. 7,977 issued September 15, 1992, and PP No. 6,906 issued July 4, 1989. U.S. Patent No. 5,330,547 issued July 19, 1994, and U.S. Patent No. 5,750,828 issued May 12, 1998, describe a method for employing *Tripsacum*-teosinte hybrids to confer corn rootworm resistance to maize.

The present invention further provides a method of screening hybrid maize seed and plants to determine if they contain introgressed DNA segments from *Tripsacum*-teosinte hybrids by isolating the total genomic DNA, digesting the DNA with restriction enzymes, transferring it to Southern blots and probing it with mapped molecular markers to determine the presence of one or more novel or unique RFLPs as defined by probe-enzyme combination and molecular weight.

The present invention provides a method for marker assisted selection of plants resistant to corn rootworm by the presence of unique DNA fragments revealed by two or more of the RFLP markers identified as UMC103, BNL5.37, UMC28, and UMC 95 and/or their SSR markers bnlg2235, dupSSR23, phi123 and bnlg1714, respectively.

The present invention provides a method for marker assisted

selection of plants with aerenchyma tissue in their roots by the presence of a unique DNA fragment revealed by the RFLP marker BNL8.32 and/or its respective SSR marker bnlg1805.

In *Tripsacum* inflorescences, the staminate (i.e. male) flowers and pistillate (i.e. female) flowers are produced on a single spike with the male flowers subtended by the female. When *Tripsacum* sends out the inflorescence, the staminate flowers are broken off leaving only the female flowers on the spike which are then covered with a pollinating bag, i.e. standard ear shoot bag for maize, to protect them from contamination by unwanted pollen. Teosinte male and female flowers occur on separate parts of the plant. The staminate flowers are borne in the tassel which emerges at the apex of the culm; whereas, the pistillate flowers occur in single-rowed spikes borne on lateral branches of the culm. When teosinte produces its tassels, they are covered with a pollinating bag. When they start shedding pollen, the bag is removed and pollen taken to pollinate the *Tripsacum* plants. At that time, the bags covering the *Tripsacum* pistillate flowers are removed and the teosinte pollen shaken out of the bag onto the silks. The *Tripsacum* inflorescence is covered again with a pollinating bag immediately after pollination and the bag is stapled so that it remains on the spike until the seed has matured. Upon maturity, approximately 45 days later, the seed is harvested. Once mature seed from the cross has been obtained, it is planted, and the plants from seed that germinates are grown in a growth chamber, greenhouse or the field. Controlled crosses are best made in a greenhouse or growth chamber where plants are kept isolated to prevent cross contamination and there is no problem with bags being damaged by weather conditions.

This method may alternatively be used to cross the plants with teosinte as the female parent. In this embodiment, all the tassels, i.e. male flowers, are removed from the perennial teosinte plant as soon as they emerge and the ears, i.e. female flowers, are covered with pollinating bags. Rather than removing *Tripsacum* male flowers, the spikes are left in tact and covered with a pollinating bag to collect *Tripsacum* pollen. The pollen is applied to the diploperennis ears which are then immediately covered with a pollinating bag that

is well fastened with staples to ensure it remains sealed until the seed has matured, approximately 45 days after pollination when the seed is harvested.

Next, when (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*) starts to flower, the same steps described above are used to cross the hybrid with maize. To cross onto maize, as soon as the maize plants begin to produce ears, before the silks emerge, the ears are covered with an ear shoot bag. Pollen collected from (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*) is applied to silks of the maize ears. The ears are then covered again with an ear shoot bag and a large pollinating bag which is wrapped around the culm and secured with a staple. The ears remain covered until they reach maturity, several weeks later when the ears are harvested.

To pollinate the (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*) hybrid with maize pollen, the tassel of the maize plant is covered with a large pollinating bag, a day or two before collection. Pistillate flowers of Tripsacum-teosinte hybrid plants frequently have staminate tips above the female flowers as described for *Tripsacum*. Whenever *Tripsacum*-teosinte plants are to be pollinated by another plant, all the staminate tips are removed as soon as the ears emerge to prevent possibility of self pollination. The pistillate flowers of the hybrid are covered with an ear shoot bag as soon as they begin to appear on the plant but before the silks emerge. Pollen collected from maize is applied to silks of the hybrid female spikes which are then immediately covered with an ear shoot bag that is stapled closed. The ears remain covered until they reach maturity, approximately 45 days later, and then the seed is harvested.

Plants obtained from all crosses described above are male and female fertile, are cross-fertile with each other, are cross-fertile with maize, and carry novel genetic material, i.e. unique polymorphisms from *Tripsacum* (see Table 3) that are not present in maize and the wild *Zeas* and novel restriction fragments (see Table 2) derived from mutations that arose in the process of intergeneric hybridization, as identified in DNA fingerprints employing 176 different molecular probes distributed throughout the ten linkage

groups of maize. Table 4 gives the molecular weights of parental RFLPs for comparative reference.

The examples and embodiments described herein are for illustration and modifications or changes that will be suggested to persons skilled in the art are to be included within the spirit and purview of this application and the scope of the appended claims.

Table 1. List of RFLP Probes Used to Fingerprint Hybrid and Parent Plants of *Tripsacum*, teosinte, *Tripsacum*-teosinte Hybrids, Maize, and Maize-*Tripsacum*-teosinte Hybrids and Derivatives.

Chromosome	1	2	3	4	5
Probe	BNL5.62	*UMC53*	UMC32	agrr115	npi409
	npi97	UMC6	asg24	*phi20725*	UMC147
	UMC157	UMC61	UMC121	UMC87	*asg73*
	UMC76	*agrr167*	BNL8.35	UMC31	*UMC90*
	UMC11	UMC34	*UMC50*	UMC55	*UMC72*
	asg45	UMC135	*UMC42*	CSU235	*UMC27*
	CSU3	UMC131	npi247	CSU585	tda66
	UMC167		UMC97	BNL5.46	UMC43
	UMC67	UMC55	UMC10	agrr321	tda37
	CSU92		UMC102	*agrr89*	UMC40
	asg62	UMC5	BNL6.06	*npi386*	BNL7.71
	UMC58		CSU240	*UMC42*	BNL5.71
	CSU164		BNL5.37	*tda62*	*tda62*
	UMC128	tda66	npi296	BNL5.71	UMC54
	UMC129		UMC60	UMC156	UMC108
	UMC107	UMC4	UMC3	UMC66	UMC68
	UMC140	UMC49	npi212	UMC19	UMC104
	adh1	UMC36	UMC39	UMC104	phi10017
	UMC161		*phi10080*	UMC133	
	BNL8.29		UMC15	UMC15	
	BNL6.32		UMC63	UMC52	
			CSU303	BNL8.23	
			UMC96	BNL15.07	
			UMC2		
			CSU25		

Table 1 (continued).

Chromosome	6	7	8	9	10
Probe	UMC85	*asg8*	npi114	phi10005	phi20075
	tda50	phi20581	BNL9.11	UMC113	BNL3.04
	npi373	O2	UMC103	UMC192	npi285
	tda204	asg34	UMC124	UMC105	KSU5
	UMC59	BNL15.40	tda52	CSU147	UMC130
	npi393	UMC5	tda164	BNL5.10	UMC64
	UMC65	UMC116	UMC32	UMC114	UMC152
	tda51	tda37	UMC120	UMC95	phi06005
	UMC21	*UMC110*	UMC89	asg44	tda205
	UMC46	tda66	BNL12.30	CSU61	
	UMC132	BNL8.32	UMC30	BNL7.57	*UMC163*
	asg7	BNL14.07	*UMC48*	BNL5.09	UMC44
	UMC28	*UMC80*	*UMC53*	CSU54	BNL10.13
	UMC62	*BNL16.06*	npi268	npi97	npi306
	UMC134	phi20020	npi414	UMC94	
			UMC7		
			UMC3		

Other Probes

Mitochondrial		Locus unknown
Probe	pmt1	tda16
	pmt2	tda17
	pmt3	tda48
	pmt4	tda53
	pmt5	tda80
	pmt6	*tda168*
		tda250

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte													
Chr m. 1	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
BNL5.62-ERI	10.3kb						10.3kb	10.3kb	10.3kb									
npi97-H	3.9kb		3.9kb				3.9kb	3.9kb	3.9kb	3.9kb				3.9kb	3.9kb			
UMC157-ERI	6.5kb		6.5kb			6.5kb												
UMC157-ERI	3.3kb		3.3kb			3.3kb												
UMC157-H	5.5kb		5.5kb			5.5kb												
UMC157-B	14.0kb	14.0kb	14.0kb	14.0kb	14.0kb	14.0kb			14.0kb									
UMC157-B		5.0kb		5.0kb					5.0kb					5.0kb				
UMC157-B			4.5kb	4.5kb			4.5kb					4.5kb			4.5kb			
UMC11-B	7.0kb	7.0kb	7.0kb	7.0kb			7.0kb	7.0kb		7.0kb		7.0kb						
CSU3-B			10.0kb	10.0kb			10.0kb	10.0kb			10.0kb	10.0kb						
CSU3-B			7.6kb	7.6kb			7.6kb	7.6kb			7.6kb	7.6kb						
CSU3-B	3.5kb		3.5kb							3.5kb		3.5kb						
UMC67-ERI				19.2kb														
UMC67-H				23.1kb														
UMC67-B	13.4kb																	
UMC67-B	11.0kb	11.0kb	11.0kb	11.0kb			11.0kb	11.0kb	11.0kb	11.0kb	11.0kb	11.0kb						
UMC67-B				1.6kb														
CSU92-B	13.3kb		13.3kb	13.3kb			13.3kb	13.3kb				13.3kb						
CSU92-B	7.5kb	7.5kb																
asg62-B	12.7kb	12.7kb	12.7kb															
asg62-B		9.7kb	9.7kb	9.7kb			9.7kb	9.7kb	9.7kb		9.7kb							
asg62-B	6.6kb		6.6kb															
UMC58-H	3.3kb		3.3kb	3.3kb	3.3kb	3.3kb												
CSU164-ERI	9.0kb		9.0kb	9.0kb			9.0kb	9.0kb			9.0kb							
CSU164-ERI		7.0kb																
UMC128-H			6.0kb	6.0kb			6.0kb	6.0kb			6.0kb	6.0kb						
UMC107-ERI			6.3kb	6.3kb	6.3kb													
UMC107-ERI			6.1kb				6.1kb	6.1kb		6.1kb								
UMC140-ERI	4.9kb		4.9kb	4.9kb			4.9kb	4.9kb	4.9kb			4.9kb				4.9kb		
UMC140-H	6.5kb		6.5kb															
adh1-H	9.4kb		9.4kb	9.4kb														
adh1-B	9.4kb		9.4kb	9.4kb	9.4kb	9.4kb												
UMC161-H	3.3kb		3.3kb	3.3kb	3.3kb	3.3kb												

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte												
Chr m. 1	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
BNL8.29-H			9.3kb								9.3kb							
BNL8.29-H	8.3kb	8.3kb	8.3kb	8.3kb								8.3kb						
Chrom. 2																		
UMC53-ERI	9.4kb		9.4kb	9.4kb	9.4kb													
UMC53-ERV		3.8kb	3.8kb	3.8kb			3.8kb	3.8kb	3.8kb			3.8kb						
UMC53-ERV	3.0kb																	
UMC6-ERI			3.8kb															
UMC6-H			9.4kb	9.4kb	9.4kb													
UMC6-B			15.3kb															
UMC6-B	13.2kb	13.2kb																
UMC6-B			12.7kb															
UMC6-B		10.0kb																
UMC6-B	7.0kb	7.0kb					7.0kb	7.0kb	7.0kb	7.0kb	7.0kb		7.0kb					
UMC61-H			3.4kb	3.4kb														
UMC61-H	2.8kb		2.8kb	2.8kb			2.8kb	2.8kb	2.8kb	2.8kb	2.8kb	2.8kb						
UMC34-ERI	7.5kb																	
UMC34-ERI	5.4kb		5.4kb	5.4kb	5.4kb													
UMC34-H	8.8kb		8.8kb	8.8kb	8.8kb													
UMC34-H	6.5kb		6.5kb															
UMC34-H	5.8kb	5.8kb					5.8kb	5.8kb		5.8kb	5.8kb	5.8kb						
UMC34-B			9.4kb	9.4kb	9.4kb													
UMC135-H		11.6kb																
UMC135-H			10.8kb				10.8kb	10.8kb		10.8kb								
UMC131-ERI			10.6kb															
UMC131-ERI	5.8kb									5.8kb	5.8kb							
UMC131-ERI	4.3kb	4.3kb	4.3kb	4.3kb	4.3kb	4.3kb	4.3kb	4.3kb	4.3kb									
UMC55-ERI	3.9kb	3.9kb	3.9kb				3.9kb	3.9kb		3.9kb	3.9kb	3.9kb						
UMC55-H	4.3kb		4.3kb															
UMC5-ERI	5.4kb		5.4kb	5.4kb	5.4kb													
UMC5-H			6.5kb	6.5kb	6.5kb													
UMC49-B		8.2kb	8.2kb	8.2kb														
UMC36-B	4.2kb																	

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte													
Chrom. 3	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
UMC32-ERI	5.3kb		5.3kb	5.3kb	5.3kb	5.3kb	6.7kb	6.7kb		6.7kb	6.7kb	6.7kb						
UMC32-H	6.7kb		6.7kb	6.7kb														
UMC32-H			6.0kb													6.0kb		
UMC32-H			2.8kb															
asg24-H	7.2kb	7.2kb	7.2kb	7.2kb					7.2kb	7.2kb	7.2kb	7.2kb						
asg24-H	6.4kb		6.4kb	6.4kb						6.4kb		6.4kb						
UMC121-ERI	3.7kb	3.7kb	3.7kb	3.7kb				3.7kb	3.7kb			3.7kb				3.7kb	3.7kb	
UMC121-ERI	3.2kb	3.2kb	3.2kb	3.2kb										3.2kb				
BNL8.35-H			9.9kb															
BNL8.35-H		8.7kb																
UMC50-B	6.8kb																	
UMC50-B			3.8kb						3.8kb	3.8kb	3.8kb		3.8kb					
UMC42-H	10.4kb	10.4kb	10.4kb	10.4kb			10.4kb	10.4kb	10.4kb	10.4kb	10.4kb	10.4kb						
UMC42-H			9.2kb	9.2kb										9.2kb	9.2kb			
UMC42-H		8.9kb		8.9kb					8.9kb									
UMC42-H			7.9kb											7.9kb		7.9kb	7.9kb	
UMC42-H	3.7kb		3.7kb				3.7kb	3.7kb	3.7kb	3.7kb	3.7kb	3.7kb						
UMC42-H	3.0kb																	
npi247-ERI	8.0kb		8.0kb	8.0kb	8.0kb													
npi247-H			3.0kb	3.0kb	3.0kb													
UMC10-ERI			6.5kb	6.5kb	6.5kb													
UMC10-ERI			5.5kb	5.5kb	5.5kb													
UMC10-H	3.0kb			3.0kb														
UMC102-ERI		2.7kb		2.7kb					2.7kb									
BNL6.06-ERI	6.8kb	6.8kb	6.8kb	6.8kb				6.8kb	6.8kb			6.8kb						
CSU240-ERI			10.6kb	10.6kb														
CSU240-ERI			4.5kb	4.5kb														
CSU240-ERI			3.3kb	3.3kb										3.3kb		3.3kb		3.3kb
BNL5.37-H			10.3kb	10.3kb			10.3kb	10.3kb	10.3kb	10.3kb	10.3kb	10.3kb						
BNL5.37-H	5.8kb	5.8kb	5.8kb	5.8kb					5.8kb			5.8kb						
BNL5.37-H	3.5kb	3.5kb	3.5kb	3.5kb			3.5kb	3.5kb	3.5kb	3.5kb	3.5kb	3.5kb						
npi296-ERI	7.9kb		7.9kb	7.9kb	7.9kb	7.9kb												
UMC3-ERI	2.5kb		2.5kb	2.5kb			2.5kb	2.5kb		2.5kb	2.5kb	2.5kb						

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte													
Chrom. 3	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
UMC3-ERI	2.0kb	2.0kb		2.0kb														
npi212-H			4.3kb	4.3kb														
npi212-B			5.4kb	5.4kb														
UMC39-ERI	12.2kb	12.2kb							12.2kb									
UMC39-ERI		9.2kb		9.2kb					9.2kb									
UMC39-ERI	7.8kb	7.8kb		7.8kb														
UMC39-ERI	7.1kb		7.1kb	7.1kb				7.1kb		7.1kb	7.1kb	7.1kb						
CSU303-ERI			10.0kb	10.0kb												10.0kb		
UMC63-H				9.5kb			9.5kb		9.5kb	9.5kb	9.5kb	9.5kb						
UMC63-H	4.3kb	4.3kb		4.3kb					4.3kb									
UMC96-H			11.8kb	11.8kb			11.8kb	11.8kb										
UMC96-H				6.4kb					6.4kb									
UMC96-H	5.5kb		5.5kb	5.5kb														
UMC96-B	7.5kb																	
UMC2-ERI	11.8kb		11.8kb							11.8kb	11.8kb							
UMC2-ERI				10.4kb														
UMC2-ERI		8.0kb		8.0kb					8.0kb									
UMC2-ERI				3.9kb														
CSU25-H	4.5kb									4.5kb	4.5kb	4.5kb						
Chrom. 4																		
agrr115-ERI			8.0kb	8.0kb	8.0kb	8.0kb												
agrr115-ERI	5.4kb		5.4kb		5.4kb	5.4kb												
agrr115-H			19.2kb	19.2kb	19.2kb													
agrr115-B			5.4kb	5.4kb	5.4kb	5.4kb												
agrr115-B			3.5kb	3.5kb	3.5kb	3.5kb												
phi20725-ERI			10.3kb	10.3kb								10.3kb	10.3kb					
phi20725-ERI		7.2kb		7.2kb				7.2kb										
phi20725-H	1.5kb									1.5kb	1.5kb							
UMC31-ERI			5.8kb	5.8kb	5.8kb													
UMC31-ERI			2.0kb	2.0kb									2.0kb		2.0kb			
UMC55-ERI	3.9kb	3.9kb	3.9kb				3.9kb	3.9kb		3.9kb	3.9kb	3.9kb						
UMC55-H	4.3kb		4.3kb															
CSU235-H			6.8kb	6.8kb										6.8kb		6.8kb		

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte												
Chrom. 4	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
CSU235-H			3.0kb													3.0kb		
CSU585-H			8.3kb	8.3kb												8.3kb		
CSU585-H			6.1kb	6.1kb														
BNL5.46-H	13.7kb																	
BNL5.46-H		10.5kb		10.5kb														
BNL5.46-H	9.7kb		9.7kb	9.7kb			9.7kb	9.7kb	9.7kb	9.7kb	9.7kb	9.7kb						
BNL5.46-H			5.1kb						5.1kb									
agrr321-B			5.5kb	5.5kb										5.5kb	5.5kb	5.5kb	5.5kb	5.5kb
npi386-H	9.3kb	9.3kb																
npi386-H	8.2kb		8.2kb							8.2kb								
UMC42-H	19.2kb		19.2kb	19.2kb	19.2kb	19.2kb												
UMC42-H	10.3kb	10.3kb	10.3kb	10.3kb			10.3kb	10.3kb	10.3kb	10.3kb	10.3kb	10.3kb	10.3kb					
UMC42-H		8.9kb		8.9kb					8.9kb									
UMC42-H	3.7kb		3.7kb				3.7kb	3.7kb	3.7kb	3.7kb	3.7kb	3.7kb						
UMC42-H	3.0kb																	
tda62-B	5.5kb	5.5kb	5.5kb							5.5kb								
tda62-B			5.2kb													5.2kb	5.2kb	
tda62-B	4.8kb		4.8kb	4.8kb														
BNL5.71-ERV	11.3kb	11.3kb	11.3kb	11.3kb								11.3kb						
BNL5.71-ERV		6.8kb		6.8kb					6.8kb									
BNL5.71-ERV	5.7kb		5.7kb				5.7kb	5.7kb		5.7kb	5.7kb	5.7kb						
UMC156-H	3.0kb		3.0kb	3.0kb	3.0kb													
UMC66-ERI			10.5kb	10.5kb														
UMC66-B	3.7kb	3.7kb	3.7kb	3.7kb			3.7kb	3.7kb	3.7kb	3.7kb	3.7kb	3.7kb						
UMC66-B			2.4kb	2.4kb										2.4kb	2.4kb	2.4kb	2.4kb	
UMC19-B	12.3kb	12.3kb	12.3kb	12.3kb			12.3kb	12.3kb	12.3kb		12.3kb	12.3kb						
UMC104-H				12.4kb														
UMC104-H	11.6kb		11.6kb															
UMC104-H		7.5kb																
UMC104-B	9.4kb		9.4kb	9.4kb	9.4kb	9.4kb												
UMC133-H	10.6kb	10.6kb	10.6kb															
UMC133-H				9.9kb														
UMC133-H			9.2kb					9.2kb	9.2kb	9.2kb	9.2kb							

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte												
Chrom. 4	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
UMC133-H				7.7kb														
UMC52-B			8.7kb	8.7kb			8.7kb	8.7kb		8.7kb	8.7kb	8.7kb						
UMC52-B	6.9kb																	
UMC52-B	3.8kb		3.8kb			3.8kb							3.8kb					
UMC52-B	3.0kb												3.0kb					
UMC52-B			2.0kb	2.0kb	2.0kb													
BNL15.07-H	2.9kb																	
BNL15.07-H				2.7kb														
Chr m. 5																		
npi409-ERI	9.4kb		9.4kb															
npi409-H			10.4kb															
npi409-H			9.0kb	9.0kb	9.0kb								9.0kb					
npi409-H	3.9kb	3.9kb	3.9kb	3.9kb		3.9kb	3.9kb	3.9kb	3.9kb	3.9kb	3.9kb	3.9kb	3.9kb					
npi409-H	3.0kb		3.0kb	3.0kb	3.0kb	3.0kb							3.0kb					
npi409-B			19.2kb	19.2kb	19.2kb													
UMC147-H		16.3kb		16.3kb			16.3kb		16.3kb									
UMC147-H	3.8kb																	
UMC147-H	2.4kb		2.4kb				2.4kb	2.4kb		2.4kb								
UMC90-H			6.5kb	6.5kb											6.5kb	6.5kb		
UMC90-H	2.8kb																	
UMC90-H	1.6kb																	
UMC90-B			9.0kb	9.0kb	9.0kb													
UMC107-ERI	6.3kb			6.3kb					6.3kb									
UMC27-H	4.5kb																	
UMC27-B	6.5kb																	
tda37-B	8.0kb									8.0kb								
tda37-B	6.4kb									6.4kb								
UMC43-B		9.7kb		9.7kb					9.7kb									
UMC43-B	5.7kb																	
UMC40-B	7.2kb																	
UMC40-B		4.7kb	4.7kb	4.7kb			4.7kb							4.7kb		4.7kb		4.7kb
UMC40-B			4.3kb	4.3kb			4.3kb				4.3kb	4.3kb						
BNL7.71-H			10.6kb								10.6kb	10.6kb						

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte													
Chr m. 5	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
BNL5.71-B	11.3kb	11.3kb	11.3kb	11.3kb								11.3kb						
BNL5.71-B		6.8kb		6.8kb					6.8kb									
BNL5.71-B	5.7kb		5.7kb				5.7kb	5.7kb		5.7kb	5.7kb	5.7kb						
tda62-B			6.5kb	6.5kb					6.5kb							6.5kb		
tda62-B	5.5kb	5.5kb	5.5kb							5.5kb								
UMC68-H	6.0kb	6.0kb	6.0kb	6.0kb			6.0kb	6.0kb	6.0kb		6.0kb	6.0kb						
UMC104-H				12.4kb														
UMC104-H	11.6kb		11.6kb															
UMC104-H		7.5kb																
UMC104-B	9.4kb		9.4kb	9.4kb	9.4kb	9.4kb							9.4kb					
phi10017-B			15.1kb															
phi10017-B	9.5kb	9.5kb	9.5kb	9.5kb			9.5kb	9.5kb	9.5kb		9.5kb							
Chr m. 6																		
tda50-B	8.5kb	8.5kb	8.5kb	8.5kb				8.5kb	8.5kb	8.5kb	8.5kb	8.5kb		8.5kb		8.5kb		8.5kb
npi373-H	6.5kb		6.5kb	6.5kb			6.5kb	6.5kb		6.5kb	6.5kb	6.5kb						
npi373-H		5.6kb		5.6kb			5.6kb	5.6kb	5.6kb					5.6kb				
npi373-H			5.1kb	5.1kb												5.1kb		5.1kb
npi373-H	3.0kb													3.0kb		3.0kb	3.0kb	3.0kb
tda204-B			4.0kb				4.0kb	4.0kb				4.0kb				4.0kb		
NPI393-ERI	12.1kb		12.1kb				12.1kb	12.1kb		12.1kb	12.1kb							
NPI393-ERI	8.5kb	8.5kb	8.5kb	8.5kb					8.5kb			8.5kb						
NPI393-ERI	5.6kb	5.6kb																
UMC65-H	2.9kb																	
UMC46-ERI	6.5kb		6.5kb	6.5kb	6.5kb	6.5kb								6.5kb	6.5kb			
UMC46-ERI	5.6kb	5.6kb	5.6kb	5.6kb			5.6kb	5.6kb	5.6kb	5.6kb	5.6kb	5.6kb		5.6kb	5.6kb	5.6kb		5.6kb
asg7-H	6.3kb																	
UMC28-H			15.8kb	15.8kb														
UMC28-H			11.9kb	11.9kb														
UMC28-B			7.6kb	7.6kb			7.6kb	7.6kb	7.6kb	7.6kb	7.6kb	7.6kb						
UMC28-B			6.6kb	6.6kb			6.6kb											
UMC134-ERI	15.3kb		15.3kb	15.3kb	15.3kb	15.3kb												
UMC134-H	7.5kb		7.5kb	7.5kb	7.5kb	7.5kb												
UMC134-H	4.7kb	4.7kb	4.7kb	4.7kb					4.7kb							4.7kb		

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte													
Chr m. 7	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
asg8-H	10.8kb		10.8kb															
asg8-H		8.4kb	8.4kb	8.4kb														
phi20581-H			4.2kb	4.2kb												4.2kb		
O2-ERI	9.4kb		9.4kb	9.4kb									9.4kb					
asg34-H			4.5kb											4.5kb		4.5kb		
BNL15.40-H	5.8kb	5.8kb	5.8kb								5.8kb							
UMC116-ERI	9.5kb	9.5kb	9.5kb	9.5kb							9.5kb							
UMC116-H	15.3kb																	
UMC110-B	10.6kb	10.6kb	10.6kb	10.6kb			10.6kb	10.6kb	10.6kb	10.6kb	10.6kb	10.6kb						
UMC110-B	4.9kb	4.9kb	4.9kb	4.9kb			4.9kb	4.9kb		4.9kb	4.9kb							
BNL8.32-H			8.9kb				8.9kb		8.9kb			8.9kb						
BNL8.32-H	7.4kb																	
BNL8.32-H		7.1kb	7.1kb	7.1kb														
BNL14.07-ERI		6.4kb		6.4kb														
UMC80-H	2.4kb	2.4kb		2.4kb				2.4kb	2.4kb									
BNL16.06-ERI	6.8kb	6.8kb	6.8kb	6.8kb				6.8kb	6.8kb			6.8kb						
BNL16.06-H	5.7kb	5.7kb	5.7kb	5.7kb			5.7kb		5.7kb					5.7kb	5.7kb	5.7kb	5.7kb	5.7kb
BNL16.06-H			3.0kb	3.0kb														
BNL16.06-H	1.6kb	1.6kb	1.6kb	1.6kb			1.6kb	1.6kb	1.6kb	1.6kb	1.6kb	1.6kb		1.6kb	1.6kb	1.6kb		
phi20020-H	7.8kb	7.8kb	7.8kb	7.8kb			7.8kb	7.8kb	7.8kb	7.8kb	7.8kb	7.8kb		7.8kb	7.8kb	7.8kb		7.8kb
phi20020-H	6.6kb																	
phi20020-H			5.1kb	5.1kb														
Chr m. 8																		
npi114-H			10.0kb	10.0kb						10.0kb	10.0kb	10.0kb						
npi114-H	8.8kb		8.8kb	8.8kb				8.8kb	8.8kb			8.8kb						
npi114-H			6.3kb									6.3kb						
BNL9.11-H		3.4kb																
UMC103-H		6.9kb	6.9kb	6.9kb			6.9kb	6.9kb	6.9kb	6.9kb	6.9kb	6.9kb	6.9kb					
UMC124-H			8.0kb	8.0kb	8.0kb	8.0kb												
UMC124-H	7.0kb		7.0kb			7.0kb												
UMC124-B	21.0kb		21.0kb	21.0kb	21.0kb	21.0kb												
UMC124-B	19.0kb		19.0kb	19.0kb	19.0kb	19.0kb												
UMC124-B			6.6kb	6.6kb														

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte													
Chrom. 8	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
UMC124-B		2.6kb	2.6kb	2.6kb			2.6kb	2.6kb				2.6kb						
UMC124-B	1.6kb	1.6kb	1.6kb	1.6kb			1.6kb	1.6kb	1.6kb	1.6kb	1.6kb	1.6kb						
UMC120-H			8.0kb	8.0kb	8.0kb	8.0kb												
UMC120-H	3.2kb		3.2kb	3.2kb			3.2kb		3.2kb	3.2kb	3.2kb	3.2kb						
UMC120-H	2.3kb																	
UMC120-H		1.4kb	1.4kb	1.4kb								1.4kb						
UMC120-B			23.1kb															
UMC89-ERI				7.3kb														
UMC89-H				7.3kb														
UMC89-B			9.5kb	9.5kb	9.5kb		9.5kb	9.5kb				9.5kb						
UMC89-B		6.0kb		6.0kb					6.0kb									
UMC89-B	5.2kb		5.2kb							5.2kb	5.2kb	5.2kb						
UMC89-B	4.5kb		4.5kb	4.5kb						4.5kb	4.5kb	4.5kb						
UMC89-MspI			6.7kb	6.7kb										6.7kb				
UMC89-MspI			5.8kb	5.8kb										5.8kb		5.8kb	5.8kb	5.8kb
BNL12.30-ERI		3.5kb																
UMC48-H			5.3kb	5.3kb										5.3kb		5.3kb		
UMC48-H				4.7kb			4.7kb		4.7kb									
UMC48-H			4.2kb	4.2kb										4.2kb		4.2kb	4.2kb	
UMC48-H			3.5kb	3.5kb			3.5kb	3.5kb				3.5kb						
UMC48-H	2.2kb																	
UMC53-ERI		3.8kb	3.8kb	3.8kb			3.8kb	3.8kb	3.8kb			3.8kb						
UMC53-ERI	3.0kb																	
npi268-B	6.4kb	6.4kb	6.4kb	6.4kb			6.4kb	6.4kb	6.4kb	6.4kb	6.4kb	6.4kb						
UMC3-ERI	2.5kb		2.5kb	2.5kb			2.5kb	2.5kb		2.5kb	2.5kb	2.5kb						
UMC3-ERI	2.0kb	2.0kb		2.0kb														
Chr m. 9																		
phi10005-ERI	15.0kb	15.0kb	15.0kb	15.0kb			15.0kb	15.0kb	15.0kb	15.0kb	15.0kb							
phi10005-ERI			1.6kb	1.6kb														
UMC113-ERI	5.9kb																	
UMC113-ERI			5.4kb	5.4kb														
UMC113-B			12.8kb															
UMC113-B		11.8kb																

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte													
Chr m. 9	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
UMC113-B	10.5kb	10.5kb	10.5kb	10.5kb			10.5kb	10.5kb	10.5kb	10.5kb	10.5kb	10.5kb						
UMC192-H	11.4kb	11.4kb	11.4kb	11.4kb			11.4kb	11.4kb	11.4kb	11.4kb	11.4kb	11.4kb						
UMC192-H		6.4kb		6.4kb					6.4kb									
UMC105-ERI			3.9kb	3.9kb														
wx-H	21.0kb		21.0kb															
CSU147-H	5.9kb	5.9kb		5.9kb														
BNL5.10-H	6.1kb	6.1kb	6.1kb								6.1kb							
BNL5.10-H	4.4kb			4.4kb														
UMC114-B	15.0kb		15.0kb	15.0kb														
UMC114-B			12.6kb								12.6kb		12.6kb					
UMC114-B	11.5kb			11.5kb														
UMC114-B			10.0kb	10.0kb			10.0kb	10.0kb		10.0kb		10.0kb						
UMC114-B	8.8kb	8.8kb		8.8kb					8.8kb									
UMC114-B			7.5kb	7.5kb				7.5kb		7.5kb				7.5kb		7.5kb		
UMC114-B	6.5kb	6.5kb		6.5kb														
UMC95-ERI	13.3kb		13.3kb			13.3kb												
UMC95-ERI	5.6kb		5.6kb															
UMC95-H			7.7kb						7.7kb									
UMC95-H			7.3kb	7.3kb										7.3kb		7.3kb		
UMC95-H	4.8kb	4.8kb	4.8kb				4.8kb		4.8kb									
UMC95-H			4.5kb	4.5kb												4.5kb	4.5kb	
UMC95-H			4.1kb	4.1kb			4.1kb	4.1kb	4.1kb	4.1kb	4.1kb	4.1kb						
UMC95-H			1.7kb	1.7kb														
UMC95-B			15.0kb	15.0kb														
UMC95-B			9.0kb	9.0kb														
asg44-ERI			5.3kb	5.3kb										5.3kb		5.3kb		
CSU61-ERI	8.1kb	8.1kb	8.1kb	8.1kb			8.1kb	8.1kb	8.1kb		8.1kb	8.1kb						
CSU61-ERI	4.8kb		4.8kb							4.8kb		4.8kb						
BNL7.57-ERI	1.0kb		1.0kb															
BNL7.57-B	11.6kb																	
BNL7.57-B		5.9kb		5.9kb			5.9kb	5.9kb	5.9kb									
CSU54-ERI	14.7kb	14.7kb		14.7kb					14.7kb									
CSU54-ERI			12.6kb					12.6kb		12.6kb	12.6kb	12.6kb						

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte													
Chrom. 9	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
npi97-H	3.9kb		3.9kb				3.9kb	3.9kb	3.9kb	3.9kb								
Chrom. 10																		
phi20075-ERI	7.1kb	7.1kb		7.1kb					7.1kb									
npi285-ERI	15.3kb		15.3kb															
npi285-ERI	12.4kb		12.4kb							12.4kb	12.4kb							
npi285-ERI	9.4kb																	
npi285-ERI		6.0kb		6.0kb					6.0kb									
KSU5-ERI	9.8kb	9.8kb		9.8kb														
KSU5-ERI		7.6kb																
KSU5-ERI		6.1kb																
KSU5-ERI		3.8kb																
KSU5-ERI	3.5kb		3.5kb							3.5kb	3.5kb	3.5kb						
UMC130-ERI		13.5kb		13.5kb														
UMC130-ERI	7.0kb		7.0kb	7.0kb			7.0kb	7.0kb	7.0kb	7.0kb	7.0kb	7.0kb						
UMC130-H	4.8kb		4.8kb	4.8kb			4.8kb	4.8kb	4.8kb	4.8kb	4.8kb	4.8kb						
UMC130-H	3.2kb		3.2kb	3.2kb					3.2kb									
UMC130-B	3.2kb																	
UMC64-H	3.3kb		3.3kb															
UMC152-H	12.4kb																	
UMC152-H	7.1kb		7.1kb							7.1kb	7.1kb							
UMC152-H		5.6kb	5.6kb	5.6kb			5.6kb	5.6kb	5.6kb			5.6kb						
phi06005	12.8kb		12.8kb	12.8kb			12.8kb	12.8kb		12.8kb	12.8kb	12.8kb						
UMC163-H			12.0kb	12.0kb														
UMC163-H	7.0kb			7.0kb					7.0kb									
UMC163-H		4.8kb	4.8kb	4.8kb			4.8kb	4.8kb	4.8kb	4.8kb	4.8kb							
UMC163-H				3.0 kb									3.0kb					
UMC163-H			2.3 kb											2.3 kb				
UMC44-H		9.8kb																
UMC44-H			8.7kb	8.7kb							8.7kb	8.7kb						
UMC44-H	7.2kb	7.2kb																
UMC44-H			5.5kb	5.5kb			5.5kb	5.5kb		5.5kb	5.5kb	5.5kb		5.5kb				
UMC44-H	4.0kb													4.0kb	4.0kb	4.0kb		4.0kb

Table 2. Novel RFLPS in *Tripsacum* -teosinte Hybrids and Maize X (*Tripsacum* -teosinte)

Probe/Enzyme	*Tripsacum-teosinte Hybrids*				Maize X *Tripsacum* -teosinte													
Chr m. 10	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**64SS**	**64TC**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**7024**	**9094**	**97-5**	**V70**
BNL10.13-H	10.8kb	10.8kb	10.8kb	10.8kb					10.8kb					10.8kb				
npi306-H				7.0kb														
Mitochondria																		
pmt1-H				2.3kb														
pmt2-H	8.0kb	8.0kb	8.0kb	8.0kb								8.0kb						
pmt2-H		4.2kb																
pmt2-H	2.8kb	2.8kb	2.8kb	2.8kb					2.8kb	2.8kb	2.8kb							
pmt2-H	2.1kb																	
pmt5-H	12.3kb	12.3kb	12.3kb	12.3kb														
pmt5-H		8.1kb												8.1kb				
pmt5-H		3.2kb		3.2kb										3.2kb	3.2kb			
pmt5-H	2.5kb		2.5kb	2.5kb								2.5kb						
Unknown																		
tda16-H				4.3kb														
tda17-H	7.0kb	7.0kb	7.0kb				7.0kb	7.0kb	7.0kb	7.0kb	7.0kb	7.0kb						
tda48-H			8.2kb	8.2kb														
tda53-H	3.8kb																	
tda53-H	2.2kb	2.2kb	2.2kb	2.2kb				2.2kb	2.2kb	2.2kb	2.2kb	2.2kb						
tda168-ERI	3.6kb			3.6kb														
tda250-B			4.0kb				4.0kb	4.0kb				4.0kb						

Table 3. Unique *Tripsacum* Alleles in *Tripsacum* -teosinte Hybrids and (Maize X *Tripsacum* -teosinte) Hybrids and Derivatives

Pr be/Enzyme	*Tripsacum* -diploperennis Hybrids				Maize X *Tripsacum* -diploperennis										
Chrom. 1	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**9094**	**97-5**	**V70**
UMC107-ERI		7.5kb													
Chrom. 2															
UMC53-ERV	8.4kb		8.4kb	8.4kb				8.4kb	8.4kb						
agrr167-B			5.7kb	5.7kb											
agrr167-B				4.5kb											
agrr167-B			4.0kb												
Chrom. 3															
UMC50-B	7.8kb	7.8kb													
UMC50-B	5.8kb		5.8kb	5.8kb	5.8kb	5.8kb		5.8kb	5.8kb	5.8kb					
UMC42-H	7.6kb			7.6kb											
phi10080-B			9.7kb	9.7kb											
CSU25-H			5.2kb	5.2kb									5.2kb	5.2kb	
CSU25-H	4.2kb	4.2kb		4.2kb			4.2kb								
Chrom. 4															
UMC31-B	6.5														
phi20725-ERI	9.7kb				9.7kb		9.7kb								
agrr89-H			7.1kb	7.1kb								7.1kb	7.1kb		7.1kb
npi386-H		12.6kb	12.6kb	12.6kb	12.6kb	12.6kb			12.6kb	12.6kb					
UMC42-H	7.6kb			7.6kb											
tda62-B	4.8kb	4.8kb				4.8kb	4.8kb	4.8kb	4.8kb						
Chrom. 5															
asg73-ERI			3.8kb	3.8kb								3.8kb	3.8kb		3.8kb
UMC90-H		7.7kb	7.7kb	7.7kb	7.7kb	7.7kb	7.7kb	7.7kb		7.7kb					
UMC72			8.5kb	8.5kb											
UMC27-H	8.3kb		8.3kb	8.3kb	8.3kb	8.3kb	8.3kb	8.3kb	8.3kb	8.3kb	8.3kb				
UMC43-B	7.3kb	7.3kb	7.3kb	7.3kb	7.3kb	7.3kb		7.3kb	7.3kb	7.3kb			7.3kb		
tda37-B	9.0kb	9.0kb	9.0kb	9.0kb	9.0kb	9.0kb	9.0kb								
UMC40-B		4.7kb	4.7kb	4.7kb	4.7kb							4.7kb	4.7kb		4.7kb
Chrom. 6															
NPI393-ERI	7.0kb	7.0kb													
UMC28-B			9.9kb						9.9kb						
Chrom. 7															
asg8-H				8.7kb											
phi20851-B			9.7kb	9.7kb											

Table 3. Unique *Tripsacum* Alleles in *Tripsacum* -teosinte Hybrids and (Maize X *Tripsacum* -teosinte) Hybrids and Derivatives

Probe/Enzyme	*Tripsacum* -diploperennis Hybrids														
Chrom. 7	**Sun Dance**	**20A**	**Tripsacorn**	**Sun Star**	**2019**	**3024**	**3028**	**3125**	**4126**	**TC64**	**Sun Devil**	**7022**	**9094**	**97-5**	**V70**
UMC110-B	3.9kb						3.9kb	3.9kb	3.9kb						
UMC80-H	10.7kb	10.7kb	10.7kb	10.7kb	10.7kb	10.7kb	10.7kb	10.7kb	10.7kb	10.7kb					
UMC80-H	8.2kb	8.2kb	8.2kb	8.2kb					8.2kb						
BNL16.06-ERI	1.9kb		1.9kb												
Chrom. 8															
UMC48-H		6.2kb		6.2kb			6.2kb								
UMC53-ERV	8.4KB		8.4KB	8.4KB				8.4KB	8.4KB						
UMC7-B	4.2kb		4.2kb					4.2kb	4.2kb						
Chrom. 10															
UMC163-H	2.6kb						2.6kb								
Mitochondria															
pmt5-H	3.6kb	3.6kb	3.6kb	3.6kb	3.6kb	3.6kb	3.6kb	3.6kb	3.6kb	3.6kb					
Unknown															
tda168-ERI	3.6kb			3.6kb											

Table 4. Restriction Fragment Sizes of Tripsacum and Teosinte Parent Plants

Probe/Enzyme	Parental RFLP Fragment Sizes	
Chromosome 1	***Tripsacum***	***Z. diploperennis***
BNL5.62-ERI	Absent	9.2kb
npi97-H	3.5kb, 3.3kb, 3.0kb, 2.8kb, 1.0kb	3.6kb, 3.4kb, 3.1kb, 1.0kb
UMC157-B	10.2kb, 3.8kb	6.7kb, 5.0kb, 3.8kb
UMC11-B	Absent	9.7kb, 7.3kb
asg45	3.2kb	1.7kb
CSU3-B	Absent	3.3kb
UMC67-B	Absent	9.8kb
CSU92-B	Absent	Absent
asg62-B	Absent	8.0kb, 5.3kb, 4.6kb, 2.4kb
UMC58-ERI	Absent	Absent
CSU164-ERI	5.7kb	6.3kb
UMC128-H	Absent	10kb
UMC107-ERI	7.9kb, 1.5kb	7.1kb
UMC140-ERI	10.9kb, 7.5kb	2.6kb
UMC161-B	10.8kb, 9.0kb	7.0kb
BNL8.29-H	Absent	Absent
Chromosome 2		
UMC53-B	8.7kb	3.8kb
UMC53-ERV	8.1kb, 5.9kb	8.8kb
UMC6-B	11.5kb, 3.3kb	11.5kb, 8.2kb, 3.3kb
UMC61-H	4.0kb	3.2kb
agrr167-B	4.0kb	4.4kb
UMC34-ERI	Absent	5.5kb
UMC135-H	12.5kb, 11.4kb	12.5kb, 11.4kb, 9.0kb
UMC131-ERI	8.5kb	10.3kb
UMC55-ERI	4.3kb, 3.7kb	3.7kb, 1.8kb
UMC5-ERI	4.9kb, 2.0kb	23.0kb, 10.0kb, 4.4kb, 1.8kb
tda66-ERI	9.1kb, 3.7kb	3.7kb
UMC4-H	4.5kb	8.0kb, 5.4kb
UMC49-B	9.4kb, 7.0kb	7.0kb, 4.2kb, 3.6kb
UMC36-B	10.5kb, 9.4kb, 8.2kb, 7.8kb, 6.6kb	3.9kb
Chromosome 3		
UMC32-H	4.2kb	9.8kb
asg24-H	4.4kb	6.8kb
UMC121-ERI	Absent	6.2kb
BNL8.35-H	3.1kb	12.2kb, 10.0kb
UMC50-B	7.1kb, 5.5kb, 3.3kb	8.2kb, 6.2kb, 3.3kb
UMC42-H	9.2kb, 7.6kb, 3.3kb, 2.7kb	3.4kb
UMC10-H	4.9kb, 2.6kb	8.1kb
UMC102-ERI	Absent	8.3kb, 6.6kb, 2.4kb, 1.4kb
BNL6.06-ERI	Absent	6.9kb, 5.7kb, 3.7kb
BNL5.37-H	Absent	9.7kb, 5.7kb
UMC3-ERI	3.3kb, 3.1kb	1.7kb
UMC39-ERI	5.7kb, 2.6kb, 1.7kb, 1.6kb	11.5kb, 7.0kb, 5.7kb, 2.5kb
UMC15-B	8.1kb, 6.6kb	5.6kb
UMC63-H	8.7kb, 7.4kb, 7.0kb, 5.9kb	14.1kb,12.5kb, 8.7kb
UMC96-H	Absent	8.7kb, 4.3kb, 3.1kb, 2.9kb
UMC2-ERI	7.2kb, 5.8kb	12.1kb
CSU25-H	9.9kb, 5.2kb, 4.0kb, 3.0kb, 2.0kb	5.9kb, 4.6kb
Chromosome 4		
phi20725-ERI	9.8kb, 5.9kb	5.9kb
phi20725-H	2.3kb	1.3kb

Table 4. Restriction Fragment Sizes of Tripsacum and Teosinte Parent Plants

Probe/Enzyme	Parental RFLP Fragment Sizes	
Chromosome 4	***Tripsacum***	***Z. diploperennis***
UMC55-ERI	4.3kb, 3.7kb	3.7kb, 1.8kb
CSU235-H	13.9kb, 9.7kb	6.3kb
CSU585-H	9.5kb, 7.1kb, 5.7kb, 3.7kb	7.6kb, 5.4kb, 4.1kb, 3.7kb, 3.0kb
BNL5.46-H	9.5kb, 6.6kb, 2.6kb. 2.3kb	9.5kb, 8.5kb, 4.0kb, 2.3kb
npi386-H	13.6kb, 12.6kb, 10.3kb	11.2kb, 9.2kb
UMC42-H	9.2kb, 7.4kb, 3.3kb, 2.7kb	3.5kb
tda62-B	4.8kb, 3.7kb, 1.8kb, 1.4kb	9.5kb, 6.7kb, 5.1kb, 4.7kb, 2.5kb, 1.4kb
BNL5.71-ERV	7.1kb, 6.6kb	6.6kb
UMC66-B	7.0kb, 3.7kb	10.5kb, 3.7kb
UMC19-B	8.5kb	10.9kb, 6.1kb
UMC104-H	Absent	7.1kb, 6.7kb
UMC133-H	Absent	4.2kb
UMC52-B	11.8kb, 5.7kb	13.9kb, 4.1kb, 3.6kb
BNL15.07-H	Absent	2.4kb
Chromosome 5		
npi409-H	13.0kb, 8.4kb, 3.0kb	13.0kb, 4.6kb
UMC147-H	Absent	2.2kb
asg73-ERI	5.9kb, 3.8kb	3.3kb, 2.4kb, 2.0kb
UMC90-H	8.4kb, 7.7kb, 5.0kb	2.4kb, 2.2kb
UMC107-ERI	7.9kb	7.1kb
UMC27-H	11.8kb, 8.0kb	5.0kb
tda37-B	9.0kb	Absent
UMC43-B	Absent	9.4kb, 7.9kb
UMC40-B	6.1kb, 5.2kb, 2.7kb	4.2kb, 3.2kb
BNL7.71-H	16.3kb, 9.0kb	10.4kb
UMC68-H	13.3kb, 5.8kb, 5.1kb, 4.3kb	5.8kb, 5.1kb
UMC104-B	Absent	7.1kb, 6.7kb
Chromosome 6		
tda50-B	10.8kb, 6.8kb, 6.6kb	8.0kb
tda50-H	1.7kb	1.4kb
npi373-H	9.0kb	9.0kb, 6.2kb
tda204-B	14.4kb, 10.5kb, 9.9kb	7.3kb, 0.9kb
NPI393-ERI	7.3kb, 5.9kb	10.7kb, 8.7kb, 5.9kb
UMC65-H	Absent	3.0kb
UMC21-ERI	Absent	5.8kb
UMC46-ERI	13.6kb, 11.9kb, 11.1kb, 8.4kb	5.9kb, 5.1kb
UMC132-H	14.0kb, 13.2kb, 11.6kb, 7.6kb, 2.0kb	13.2kb, 9.9kb, 5.4kb
asg7-H	Absent	9.7kb, 5.3kb
UMC28-H	10.0kb	5.8kb, 2.0kb
UMC28-B	14.0kb, 9.9kb	14.0kb, 4.1kb
UMC134-B	9.9kb, 2.9kb, 2.8kb, 2.7kb	4.2kb, 3.6kb
Chromosome 7		
asg8-H	9.3kb, 6.9kb	11.0kb
BNL15.40-H	6.8kb, 3.9kb, 3.2kb	10.4kb, 5.1kb
UMC116-ERI	Absent	Absent
UMC110-B	7.3kb, 6.6kb, 3.9kb	7.3kb
BNL8.32-H	12.2kb	12.2kb, 10.1kb, 7.3kb
BNL14.07-ERI	Absent	6.7kb, 5.7kb
UMC80-H	10.7kb, 8.9kb, 8.2kb, 6.2kb, 3.5kb	6.1kb, 5.4kb
BNL16.06-ERI	8.6kb, 7.2kb, 3.1kb, 2.0kb	8.6kb, 6.7kb, 3.7kb, 1.8kb
phi20020-H	12.0kb, 2.8kb	12.0kb, 8.3kb

Table 4. Restriction Fragment Sizes of Tripsacum and Teosinte Parent Plants

Probe/Enzyme	Parental RFLP Fragment Sizes	
Chromosome 8	***Tripsacum***	***Z. diploperennis***
tda18-H	7.7kb, 7.0kb, 2.9kb	6.1kb
npi114-H	5.4kb, 3.9kb	3.9kb, 1.3kb
BNL9.11-H	4.6kb, 3.3kb	3.3kb, 1.5kb
UMC103-H	Absent	11.5kb
UMC124-B	3.3kb, 3.1kb, 1.8kb	3.1kb, 2.3kb, 1.8kb, 1.1kb
UMC120-H	Absent	2.1kb, 1.5kb
UMC89-B	Absent	5.4kb, 4.6kb
BNL12.30-ERI	Absent	8.9kb
UMC48-H	6.4kb, 5.0kb, 4.0kb	8.0kb, 5.0kb
UMC53-ERI	8.7kb, 8.2kb	3.8kb
npi268-B	Absent	6.8kb, 6.2kb
UMC7-B	4.3kb, 4.1kb	3.0kb
UMC3-ERI	3.3kb, 3.2kb, 3.1kb	1.7kb
Chromosome 9		
phi10005-ERI	6.1kb	10.2kb
UMC113-ERI	7.3kb	Absent
UMC192-H	10.7kb, 9.9kb, 9.2kb, 1.7kb	8.3kb, 7.3kb, 2.1kb
CSU147-H	2.7kb, 1.6kb	5.7kb, 5.0kb
BNL5.10-H	Absent	2.5kb
UMC114-B	Absent	9.2kb, 6.7kb
UMC95-ERI	4.1kb	4.8kb, 4.1kb
CSU61-ERI	2.6kb	7.7kb, 2.6kb
BNL7.57-ERI	Absent	5.0kb, 4.4kb
CSU54-ERI	3.6kb, 1.6kb	Absent
Chromosome 10		
phi20075-ERI	1.5kb	8.3kb, 7.0kb
npi285-ERI	8.2kb, 5.6kb	7.0kb
KSU5-ERI	Absent	3.5kb, 2.2kb
UMC130-ERI	Absent	Absent
UMC130-H	Absent	8.8kb, 4.3kb
UMC152-H	Absent	7.0kb, 5.3kb
phi06005	7.2kb	10.8kb, 8.8kb
UMC163-H	6.6kb, 6.4kb, 5.7kb, 2.8kb	12.1kb, 4.6kb, 4.2kb
UMC44-H	6.4kb, 5.5kb	6.4kb, 3.2kb
BNL10.13-H	12.6kb, 9.1kb, 6.7kb, 6.0kb	3.9kb
npi306-H	2.3kb, 2.0kb	11.3kb, 9.0kb
Mitochondria		
pmt1-H	7.5kb, 6.5kb	8.4kb, 2.8kb, 2.7kb
pmt2-H	1.0kb	7.8kb, 4.2kb, 1.7kb, 1.0kb, 0.8kb
pmt3-H	2.9kb, 2.3kb	5.1kb, 2.1kb
pmt4-H	8.5kb	8.5kb, 5.5kb
pmt5-H	7.4kb, 3.8kb, 2.7kb	9.1kb, 5.9kb, 4.4kb, 3.6kb
pmt6-H	8.6kb, 1.9kb	4.8kb, 3.5kb
Locus Unknown	***Tripsacum***	***Z. diploperennis***
tda16-H	7.7kb, 6.0kb, 2.9kb	6.1kb
tda17-H	12.9kb, 8.5kb	Absent
tda48-H	13.5kb, 10.5kb, 10.3kb	13.5kb
tda53-H	6.0kb, 5.7kb, 5.0kb, 2.2kb, 1.8kb	2.2kb
tda168-ERI	4.1kb, 3.6kb, 2.5kb	4.1kb, 2.5kb
tda250-B	10.3kb, 6.5kb, 3.7kb	2.7kb

Table 5. RFLP and SSR Markers that Map to the Same Genetic Loci

RFLP Marker	*Corresponding SSR*	*RFLP Marker*	*Corresponding SSR*	*RFLP Marker*	*Corresponding SSR*	*RFLP Marker*	*Corresponding SSR*
Chromosome 1	**Chromosome 1**	**Chromosome 3**	**Chromosome 3**	**Chromosome 5**	**Chromosome 5**	**Chromosome 9**	**Chromosome 9**
BNL5.62	bnlg1124	BNL5.37	dupssr23	phi10017	bnlg389	phi10005	bnlg2122
npi97	bnlg1112	UMC60	bnlg2241	**Chromosome 6**	**Chromosome 6**	UMC113	phi122
UMC157	bnlg1953	UMC39	bnlg1182	UMC85	bnlg426	UMC105	bnlg1082
UMC76	bnlg1484	UMC63	bnlg1536	npi373	bnlg1047b	CSU147	bnlg1626
UMC11	bnlg1083	UMC103	bnlg1754	UMC59	bnlg2191	BNL5.10	bnlg127
asg45	bnlg1016	UMC96	bnlg1257	NPI393	bnlg2151	UMC114	bnlg469a
CSU3	bnlg2295	UMC2	bnlg1098	UMC65	phi124	UMC95	bnlg1714
UMC67	bnlg1273	**Chromosome 4**	**Chromosome 4**	UMC21	bnlg1922 or phi129	BNL5.09	bnlg1588
asg62	bnlg615	agrr115	bnlg372 or bnlg1370	UMC46	bnlg1702 or bnlg2249	npi97	bnlg1506
UMC58	bnlg1556	phi20725	bnlg1241	UMC132	bnlg1759a	**Chromosome 10**	**Chromosome 10**
UMC128	bnlg1629 or bnlg2228	UMC87	bnlg1126	asg7	bnlg1521	UMC130	bnlg1762
UMC107	bnlg1502 or bnlg1597	UMC31	bnlg1162	UMC28	phi123	UMC64	bnlg2336
adh1	bnlg1268	npi386	bnlg1217	**Chromosome 7**	**Chromosome 7**	phi06005	bnlg1037
UMC161	bnlg1671	UMC156	bnlg1729	asg8	bnlg2132	tda205	bnlg1074
BNL8.29	bnlg2331	UMC66	bnlg2291	phi20581	bnlg1292	UMC163	bnlg1185
Chromosome 2	**Chromosome 2**	UMC19	dupssr34	O2	bnlg1200	UMC44	bnlg1250
UMC53	bnlg1338 or phi98	UMC15	dupssr28	asg34	bnlg1094	BNL10.13	bnlg594
UMC6	bnlg125 or bnlg4696	UMC52	bnlg1019b	BNL15.40	bnlg1759b	npi306	bnlg2190
UMC61	bnlg16216	BNL8.23	bnlg1337	UMC110	bnlg572		
UMC34	bnlg1064	BNL15.07	bnlg589	BNL8.32	bnlg1805		
UMC135	bnlg166	**Chromosome 5**	**Chromosome 5**	UMC80	dupssr13		
UMC131	bnlg1831 or bnlg1909	npi409	bnlg1006	BNL16.06	bnlg23286		
UMC55	bnlg1396	UMC147	bnlg1836	phi20020	phi69		
UMC5	bnlg1413	UMC90	bnlg143 or bnlg1382	**Chromosome 8**	**Chromosome 8**		
UMC4	bnlg1233	UMC72	bnlg219	npi114	bnlg2037 or bnlg1252		
UMC49	bnlg1940	UMC27	bnlg1660	BNL9.11	bnlg1194		
Chromosome 3	**Chromosome 3**	BNL7.71	bnlg1287	UMC103	bnlg2235		
asg24	bnlg1523	BNL5/71	bnlg2323	UMC124	bnlg1067		
BNL8.35	bnlg1047a or bnlg1798	UMC54	bnlg609 or bnlg1246a	UMC120	bnlg669		
UMC10	bnlg1452	UMC108	bnlg1306	UMC89	bnlg666		
UMC102	bnlg2047	UMC68	bnlg2305				

DEPOSIT OF SEEDS

A sample comprising at least 2500 seeds derived from crosses between *Tripsacum dactyloides* and *Zea diploperennis* as described herein were deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 on August 28, 1992. The accession number is ATCC75297.

The present invention is not limited in scope by the seeds deposited, since the deposited embodiments are intended as illustrations of the invention and any seeds, cell lines, plant parts, plants derived from tissue culture or seeds which are functionally equivalent are within the scope of this invention. An adequate supply of seed from other crosses, including crosses between *Tripsacum laxum* and *Zea diploperennis,* are available for deposit with the American Type Culture Patent Depository if necessary. While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that changes and modifications can be made without departing from the spirit and scope of the invention in addition to those shown and described herein. Such modifications are intended to fall within the scope of the appended claims.